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The Chemical Composition and Analysis of Citronella Oil*

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1. *Introduction*

Citronella oil is the essential oil obtained from citronella grass which is grown predominantly in the south of Sri Lanka. The two cultivated types are known as MAHAPENGIRI and LENABATU and they are distinguished morphologically by the shape and length of their leaves[13]. The two types are considered to be derived from the same parent ; and are now botanically classified as [1,5] :—

Lenabatu	(= Heen pengiri)	:	*Cymbopogon nardus* (L) Rendle
	(Ceylon type)	=	*Andropogon nardus* Ceylon de Jong
Mahapengiri	(Java type)	:	*Cymbopogon winterianus* Jowitt
		=	*Andropogon nardus* Java de Jong

Differences in the chemical composition of the essential oils from the two varieties have been recorded since early times[5,9]. It was believed that the Mahapengiri variety contained around 85% of "total acetylisables expressed as geraniol" the chief among which were geraniol (I), citronellal (II) and citronellol (III). The Lenabatu variety on the other hand was reported to contain only 55—65% of "total acetylisables expressed as geraniol". Both types of oil were, and still are, in demand in commerce. The Lenabatu-type is used as a deodorant in mosquito repellent applications, and for the scenting of soaps, sprays, disinfectants, paints and polishes. The Mahapengiri-type is used mainly as a starting material for the preparation of industrially important perfumery compounds derived from geraniol, citronellol and citronellal, for which purpose the Lenabatu-type is not as suitable.

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* Based on a lecture delivered at the Institute of Chemistry, Ceylon, *Annual Sessions,* June 1973.

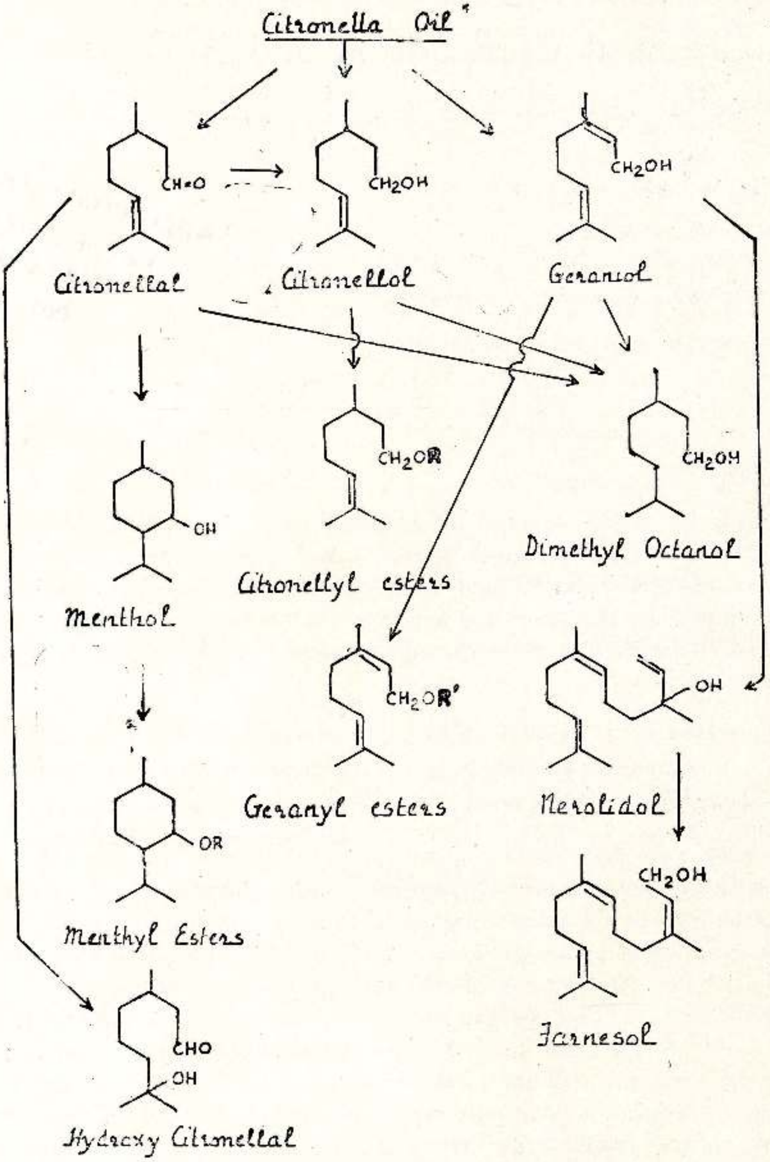


Chart 1. Synthetic perfumery compounds obtainable from Citronella Oil.

The main constituents of the two types of citronella oil had been "identified"[5] by means of classical chemical methods (*Table* 1). These classical methods were based on comparatively drastic fractional distillation procedures. Hence it was quite possible that chemical transformations took place and some at least of the products were artefacts not present in the original oil. Yet the identification and characterisation of these compounds at the time, were laudable feats of chemistry[14]. The classical methods of analysis[5] of the essential oil of citronella were primarily based on two factors : firstly, the estimation of the "total acetylisables" in them and secondly, various rough solubility checks known as, "Schimmel's test", "Raised Schimmel's test" and "London solubility test"[5]. In addition, the limiting values for various physical constants such as refractive index and optical rotation were specified.[3]

Table 1[5] Chemical Constituents of Citronella oil (Classical Methods).

Lenabatu	Mahapengiri	
Camphene	Limonene	Citronellyl oxide
Dipentene (limonene)	Citronellal	γ and δ cadinene
Citronellal	Citral	Vanillin
Geraniol	Geraniol	Isovaleraldehyde
Geranyl acetate	Citronellol	Hexene-2-al
Nerol	Geranyl butyrate	3-Methyl-pentanal
Citronellol	Citronellyl citronellate	
Thujyl alcohol	Eugenol	
Borneol	Methyl eugenol	
Farnesol	Chavicol	
Linalool	Sesquicitronellene	
Methyl eugenol	Elemol	

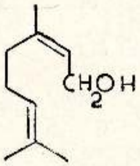
2. *The Advent of Instrumental Methods*

Two factors were mainly responsible for what may be called the "second look" at the volatile essential oils in general, from the view point of their chemical constituents. The first was a remarkable phase in the development of organic chemistry itself. The new techniques for the characterisation of chemical compounds based on spectroscopic methods resulted in a major surge in natural products research in general, during the 1960—70 decade[4]. These techniques not only afforded a deeper insight into structural features of organic molecules, but they also needed comparatively small quantities of compounds. Prior to this, the characterisation of organic compounds was dependent on several time-consuming operations ; firstly the preparation of derivatives and determination of their physical constants such as melting point, optical rotation, etc ; then the degradation or conversion to known compounds; and finally identifying the inter-relationship between the degradation products and the original whole - an exercise which was much in the nature of piecing together a complex jigsaw puzzle. These operations needed comparatively large amounts of material which were often difficult to obtain. The second factor that facilitated a re-study of essential oils was the development of new separation techniques based on chromatography. The methods of separation available previously were the comparatively drastic ones of fractional distillation and chemical reactions based on particular functional groups. Fractional distillation often caused changes due to isomerisation, polymerisation or decomposition even when carried out at reduced pressures. Separations based on chemical reactions were applicable only in comparatively few instances ; as for example, the isolation of eugenol (IV) from the other constituents of cinnamon leaf oil and clove oil, by alkali treatment. The most significant advance as far as essential oils were concerned was the development of gas-liquid chromatography (GLC). This gave a new dimension to studies on essential oils and their chemical constituents. Here was a technique that seemed from the beginning to be ideally suited to the study of essential oils. It depended much for its effectiveness on the volatility of the compounds ; and the constituents of essential oils by the very nature of their preparation are that. There were in most cases a large number of chemical compounds in essential oils, and their examination depended on the extent to which these could be effectively separated. GLC afforded a fantastic method of separation which could be achieved with very minute amounts of material. Furthermore, preparative GLC afforded a means of isolation of the separated constituents which could then be subjected to the scrutiny of new techniques, such as ir, NMR spectroscopy and mass spectrometry, or to microchemical reactions, in order to determine or ascertain their chemical nature.

The combination of GLC and infra-red (ir) spectroscopy seemed then to offer an ideal means to commence a new study on the old problems of citronella oil.

3. *The Chemical Composition of Citronella Oils*

The techniques used in the study of citronella oil[16] were similar to those used previously in a study[2] of the essential oil of cardamom varieties. They were based firstly on obtaining the maximum possible resolution on GC columns (*Figure* 1) ; and secondly, on identifying the various constituents by retention data, and peak enrichment techniques. In peak enrichment, the authentic compounds one at a time were added to the oil prior to injection. The enhancement of the peaks indicated the corresponding positions of the added substances. Such identifications however were necessarily tentative and had to be confirmed by ir spectroscopy. The individual compounds resolved by preparative GLC were collected either into pre-cooled solvents or liquified in capillary tubes cooled to below zero temperatures. Their spectra were then matched with those of authentic substances which too had been purified the same way. In this way the chemical identities of many of the constituents of citronella oil were confirmed. The main differences in chemical composition both qualitative and quantitative, between the oils of Mahapengiri and Lenabatu were also established (*Table* 2). A comparison of the GLC tracings (*Figure* 1) also



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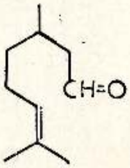

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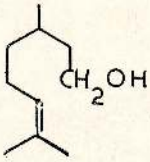

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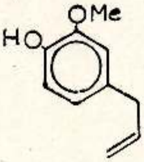

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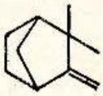

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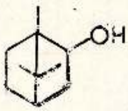

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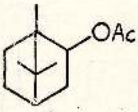


(IX) .

Operating parameters for GLC tracings in Figure 1 :

Instrument : Varian Aerograph 1740-1 with F.I.D. detector
Column : 10% Carbowax on Chromosorb W(2.7 × 3,2 mm.)
Programme rate : 60°—220°, at 2°/min., linear
Base attenuation : X 16
(Attenuations above X 16 are indicated).

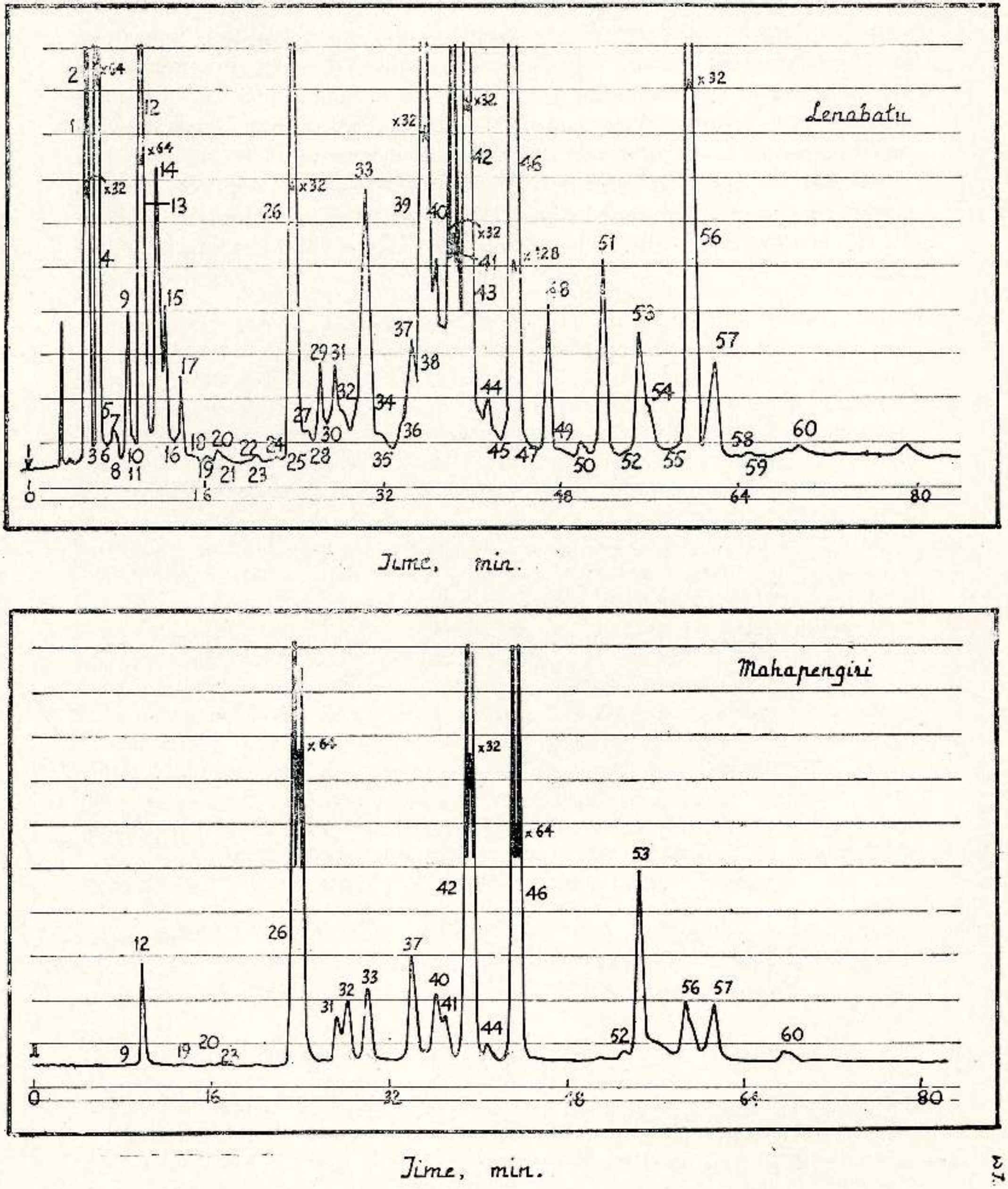


Figure 1. Comparative temperature programmed gas chromatograms of Citronella Oil (Lenabatu type) and Citronella Oil (Mahapengiri) on Carbowax 20 M. (Operating Condition vide p. 71)

Table 2[16] Chemical Constituents of Citronella oil (Instrumental methods).

Peak No.	Compound	Approximate percentage present in	
		Mahapengiri	Lenabatu
0	Solvent		
1	Tricyclene	—	1.6
2	α – Pinene	—	2.6
4	Camphene	—	8.0
5	β – Pinene	—	trace
6	Sabinene	—	trace
7	Myrcene	—	0.3
8	Car-3-ene	—	trace
9	α – Phellandrene	—	0.8
10	α – Terpinene	—	—
12	Limonene	1.3	9.7
14	*cis*-Ocimene ; γ – Terpinene	—	1.4
15	*trans*-Ocimene ; β – Phellandrene	—	1.8
16	*p* – Cymene	—	trace
17	Terpinolene	—	0.7
20	1—Hexanol	—	0.1
23	Methyl heptenone	trace	0.2
24	Unidentified	—	trace
25	Unidentified	—	trace
26	Citronellal	32.7	5.2
27	Camphor	—	0.5
28	Bourbonene	trace	1.0
29	Linalool	1.5	1.2
30	Linalyl acetate	2.0	0.8
32	α – Terpineol	—	trace
33	β – Caryophyllene	2.1	3.2
34	4 – Terpineol	trace	0.7
35	Menthol	—	trace
36	Unidentified	trace	trace
37	Citronellyl acetate	3.0	1.9
38	Unidentified	—	trace
39	1 – Borneol	trace	6.6
40	Geranyl formate	2.5	4.2
42	Citronellol ; Geranyl acetate*	15.9	8.4
44	Nerol	7.7	0.9
46	Geraniol	23.9	18.0
47	Citronellyl butyrate	trace	trace
48	Geranyl butyrate	—	1.5
50	Nerolidol	—	0.3
51	Methyl eugenol	trace	1.7
53	Elemol	6.0	1.7
56	Methyl iso-eugenol Eugenol	2.3	7.2
57	Unidentified	1.4	1.5
60	Farnesol	0.6	trace

* Minor component which merges into major peak, separate peaks are revealed at optimum loading of column.

reveals these differences. One of the striking differences observed was the presence in the Lenabatu variety of several monoterpene hydrocarbons amounting to more than 20% of the oil as against 3—4%, mainly limonene (V) in the Mahapengiri variety. The presence of a high proportion of hydrocarbons in Lenabatu oil has been recorded previously[12]. Of the monoterpene hydrocarbons in the Lenabatu variety the most abundant was camphene (VI), while the presence of another solid hydrocarbon tricyclene (VII) has been confirmed recently[16].

The other hydrocarbons present were α and β pinenes, sabinene, myrcene, car-3-ene, α and β phellandrene, α and γ—terpinene, *cis* and *trans* ocimene, terpinolene, and *p*-cymene. The frequent co-occurrence of tricyclene and camphene in natural essential oils has been previously recorded by Zavarin and Snajberk[19] in the case of *Abies* cortical oleoresin. The occurrence of these two compounds together with borneol (VIII) and bornyl acetate (IX) in citronella of Lenabatu variety is an indication that the biosynthetic pathway *via* neryl or geranyl pyrophosphate (X) and the 2-bornane carbonium ion (XI) is operative in the case of this plant. (*Chart* 2)

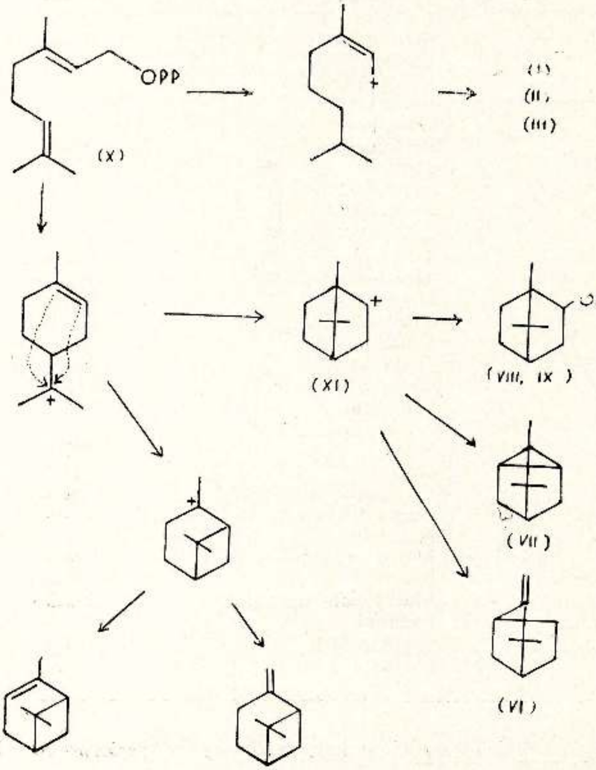
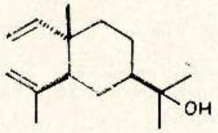


Chart 2. Possible biosynthetic pathways

The Mahapengiri oil contained more oxy-terpenes than the Lenabatu variety. There was no great difference in the amounts of geraniol in the two oils. However the Mahapengiri variety contained much more of citronellal (II) and citronellol (III) which doubtless helped to raise its level of "total acetylisables". Borneol (VIII) was only present as a trace in the Mahapengiri variety but was a significant constituent of Lenabatu-type oil. This solid was no doubt an important contributor to the particular fragrance tones for which the Lenabatu-type is used : *viz* as a soap scenting, and to its reputed "staying" qualities, when thus employed.

The occurrence of the sesquiterpene elemol (XII) has been recorded before[5] in the case of Mahapengiri oil, and its presence in Lenabatu too has been confirmed[16]. It had been observed by Jones and Sutherland[8] that elemol may not really be the compound that is present in essential oils, but that it may be formed from its thermolabile precursor hedycaryol (XIII).



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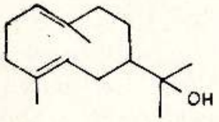

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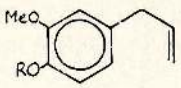

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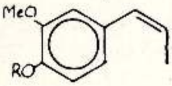

(XV)

This is in fact so in the case of citronella oil as, preparative GLC carried out isothermally at 80°C with a low injection temperature of 90° on citronella oil obtained by solvent percolation at ambient temperatures gave hedycaryol and not elemol[16]. Another distinguishing feature of the Lenabatu-type oil is the presence in it of methyl eugenol (XIV, R = Me) and methyl isoeugenol (XV, R = Me). These compounds are the major peaks which appear last on the chromatograms, and are present in only comparatively small quantities in the Mahapengiri variety.

4. *The Story of Adulteration with Kerosene*

For many years it had been assumed that citronella oil produced in Sri Lanka was adulterated with kerosene oil[5]. A publication by Guenther[6] carried this story, illustrated with a variety of anecdotes, from personal experiences of interviews and discussions with exporters in Sri Lanka;

> "There is no secret about the addition of kerosene to Ceylon oils, every exporter and most distillers are perfectly frank about it"

wrote Guenther in 1940 in this report based on a personal survey made in Sri Lanka.

The original method for the detection of kerosene in citronella oil had been devised by the analysts of Schimmel and Co., and introduced in 1898. Two tests known as "old Schimmel's test" and "new Schimmel's test" were originally available. They were both based on solubility in 1—2 volumes of 80% *v/v* ethanol, and the detection of turbidity, in a questionable sample on the further addition of ethanol. The "raised Schimmel's test" was a subsequent development where 5% kerosene was added to the fresh sample and the "old Schimmel's test" applied. Separation of an oily layer was cause for rejection of the sample of oil. The "raised Schimmel's test" is the one mostly employed here by analysts who are called upon to assess the quality of samples of citronella oil. But Guenther[6] recorded that this test, had no practical significance in the trade due to the fact that oils which were slightly old did not readily pass it. Contracts for Sri Lanka citronella oil were mostly based on the "old Schimmel's test[6]. It was felt that the "raised Schimmel's test" was sufficiently exacting to practically exclude any adulterants but there were doubts too as Guenther recorded[6] :

> "Most of the pure freshly distilled oils meet this test without any difficulty *except perhaps certain estate oils which for unknown reasons seem to be less soluble even when freshly distilled**.

Due mainly to the stigma of alleged adulteration the Sri Lanka citronella industry had almost collapsed. Doubts as to the validity of the tests used for the detection of kerosene adulteration were in fact cast by Joachim[7] as early as 1929. He found that oils freshly distilled by him from citronella grass at the Agricultural Experimental Station at Damana in the Batticaloa district and Weligama in the Matara district also failed to pass the various Schimmel's tests. Although the authenticity of these oils was unquestionable, and their content of "total acetylisables expressed as geraniol" were within the average, they were rejected on the market. This caused Joachim to undertake further investigations into the "quality" of Sri Lanka grown citronella, after which he concluded[7] as follows :

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*Italics by the present author.

> "There is evidently little relationship between the quality of Ceylon citronella oils as gauged by their geraniol contents and their response to Schimmel's test. It has been found that pure unadulterated oils containing at times high geraniol percentages do not pass the test".

Unfortunately Joachim's work had not received adequate attention. The result was that a situation had arisen where perfectly fresh and unadulterated oils were being rejected on the assumption that they were adulterated[6], merely because they failed to pass "tests" which were arbitrary, had no chemical basis, and were at best dubious safety-valves for the buyer.[6] The damage this caused to the citronella industry in Sri Lanka and the island's foreign exchange earnings, cannot be overemphasised. It was in this context that the CISIR's own programme into the chemistry of the essential oils commenced. The first requirement was to study the composition of the oils in detail and thereby develop the necessary knowledge and expertise in analytical techniques, particularly GLC, which would enable the solution of the problems of quality assessment and adulteration. The results briefly discussed earlier[15,16] were achieved as a direct consequence of this approach. The perfection of a method for detection of kerosene then followed[16]. Kerosene itself is a complex mixture of hydrocarbons (*Figure* 2). When a sample of citronella oil containing

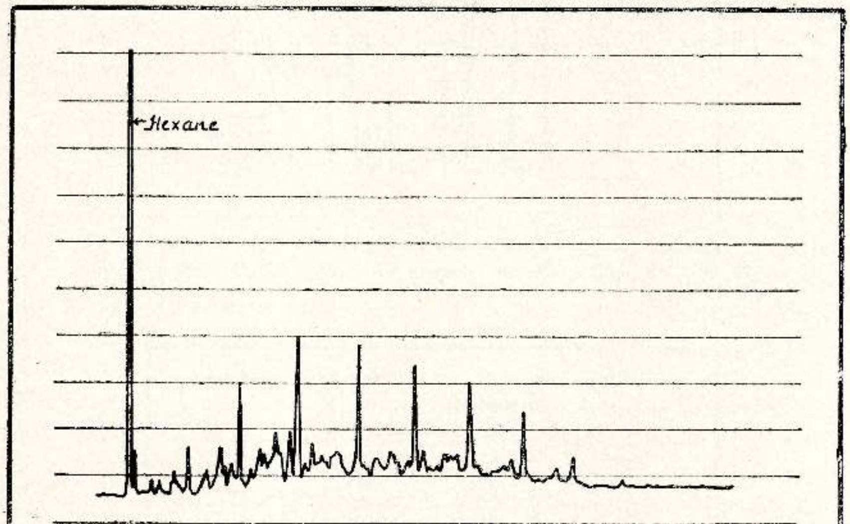

Figure 2. 50% Kerosene in Hexane

Operating Parameters for GLC tracings in Figures 2-4

Instrument	:	Varian Aerograph 1740—1.
Column	:	10% SE.30 on Chromosorb W. (1,5 m × 3,2 mm).
Programme rate	:	60° — 220° at 2°/min. linear.

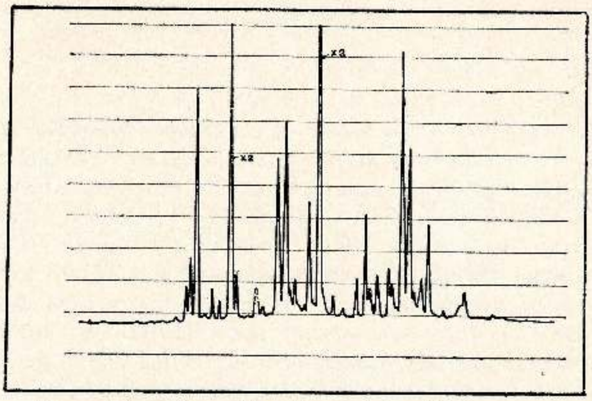

Figure 3 A. Citronella Oil containing 6% Kerosene.

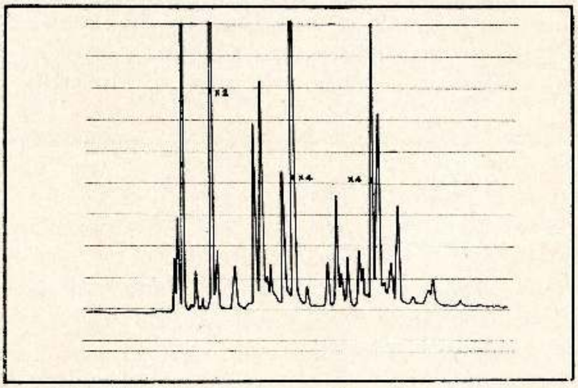

Figure 3 B. GLC profile of pure Citronella Oil (Lenabatu)

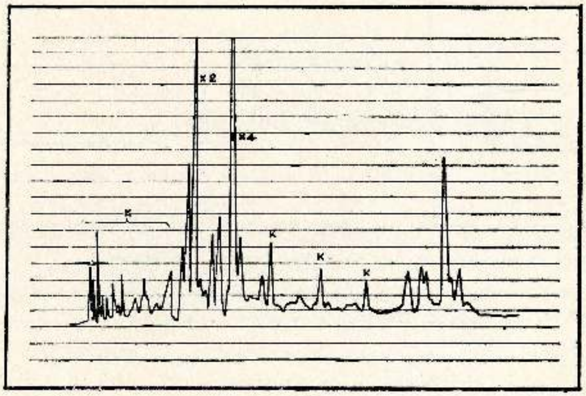

Figure 4. Citronella Oil containing 5% Kerosene, after removal of oxygenated terpenes by preferential adsorption on silica gel. Peaks marked 'K' are due to Kerosene.

kerosene is subjected to GLC the peaks due to the kerosene mostly merge into peaks due to the genuine constituents of citronella. It is thus difficult to detect any adulterant. (*Figure* 3). However when the oxygenated compounds which usually occupy the middle region of the chromatogram are removed by preferential adsorption on silica gel, the hydrocarbon peaks due to kerosene become much more prominent (*Figure* 4). This method is reproducible, and it is possible to detect as little as 2—3 per cent adulteration. It has been used during the past few years and by means of it a great many samples of citronella oil have been tested. The results appear to point to the inescapable conclusion that the practice of adulteration has now been abandoned, in Sri Lanka. No sample containing kerosene has yet been detected. The failure of freshly distilled oils to pass the various solubility tests such as "Schimmel's test" is readily explained by the presence in the Lenabatu-type oil of a very high proportion of natural terpene-hydrocarbons. In respect to solubility in alcohol, these too would react in similar fashion to kerosene hydrocarbons. The GLC method briefly described here can also be extended to determine quantitatively the extent of adulteration with kerosene (if any) up to a lower limit of 2%[18]. However the development of this method served another purpose. This was to exonerate the oil produced in Sri Lanka from alleged adulteration ; for, as Guenther had earlier advised[6] the ability to detect any malpractices at the producers end would benefit the market potential.

5. *Other Benefits of Research*

The systematic study of citronella oil in relation to chemical composition resulted in several other benefits apart from the development of analytical methods for quality assessment. It gave us a basic insight into the chemical composition of this oil, and the extent to which the main constituents varied in proportion. These results were applicable for many purposes. For instance new stills for the distillation of citronella oil were being developed by the CISIR[10,11,17].** The studies enabled the systematic monitoring, using GLC, of the performance of these stills ; the formulation of ideas on the correct methods of preparation of the plant material ; and the optimum time of harvesting of the grass. For instance, it was found that immature grass generally had an even higher content of terpene hydrocarbons than the mature ones ; and that the wilting process was necessary for the production of good quality oil. Seasonal variations are also being observed. In addition the expertise and techniques developed led to the discovery of several possible "varieties" of citronella which consistently gave oils of composition[18] different to either the

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** Under a project called IPOMEC (Industrial Products of Minor Export Crops) funded by the Ministries of : Plantation Industries *and* Planning and Employment.

Lenabatu-type or Mahapengiri-type. The morphological differences between these varieties have been recognised, and it would appear that the Lenabatu-type oil is a mixture of the oils from several of these "varieties"†. Experiments on propagating these varieties individually are now in progress,§ and it may be possible to develop varieties containing oil with a high proportion of oxygenated compounds, to suit particular market requirements abroad. The "Mana" or wild citronella varieties are also being included in this study.

6. *Acknowledgement*

The author is grateful to his colleagues, in the Natural Products Section of the Ceylon Institute of Scientific and Industrial Research (CISIR), whose work forms the basis for this paper.

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† These are now termed "chemical races".

§ In collaboration with Dr. W. Herath, Faculty of Agriculture, University of Sri Lanka (Peradeniya Campus), and sponsored by the National Science Council of Sri Lanka.

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Manioc : Selected Topics

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1. *Introduction*

The Manioc (cassava, mandioca, yuca) plant originating in tropical America, is widely cultivated in Africa, Asia and Latin America. There are over 200 cultivars of the plant and these have been classified on external morphology[81], cyanogenic glucoside content and other criteria. Workers tend to classify the varieties of the plant in different ways : *Manihot utilissima* Pohl and *Manihot esculenta* Crantz are considered to be one species by some groups[54] while others[31] class these two as different species.

Most of the data on manioc in this review has been taken from work published under these two names. They have been treated as synonyms including all varieties yielding an edible tuber.

The notion[86] that "sweet" varieties of manioc that contain *no* cyanogenic compounds are available, has not been recognised in any of the extensive studies on this aspect[40,65]. It is common to refer to "*sweet*", "*average toxic*" and "*bitter*" varieties of manioc ; these terms generally refer to those varieties in which the edible portion contains less than 50, 50—100 and more than 100 mg of cyanide per kg flesh respectively. However, this classification is basically incorrect, as the cyanide content of a single cultivar varies when it is grown under different environmental conditions[54]. Further, Sinha and Nair[89] have shown that the bitterness of tubers cannot be attributed to the concentration of cyanogenic glucosides alone.

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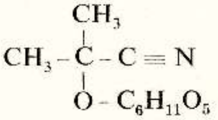
2. *Cyanogenic Glucosides of Manioc*

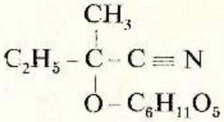
2.1. **Chemistry:** There are two cyanogenic glucosides of manioc: *Linamarin*[71] (A) and smaller amounts of *Lotaustralin*[11] (B).

(A)



$$CH_3-\overset{\displaystyle CH_3}{\underset{\displaystyle O-C_6H_{11}O_5}{C}}-C\equiv N$$

(B)



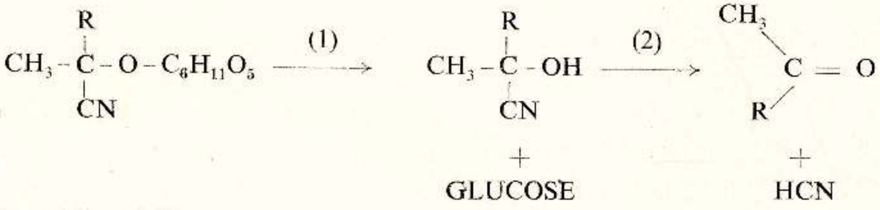
$$C_2H_5-\overset{\displaystyle CH_3}{\underset{\displaystyle O-C_6H_{11}O_5}{C}}-C\equiv N$$

In nature, linamarin was found to be the β anomer[18]. The amino acids valine and isoleucine were found to be the precursors for the aglycone moieties of the glucosides (A) and (B) respectively[70]. Methods are available for the isolation and purification of linamarin[18,99]. The method by Wood[99] has been tested by one of the authors and it was found that the glucosides can be isolated efficiently by this method (~0.3 % the wet weight of rind for local types).

The glucosides which are soluble in water, are stable to boiling but are decomposed at 150°C[17] *and* by the enzyme linamarase (linase) which acts on both linamarin and lotaustralin[70] to produce glucose, hydrogen cyanide and the corresponding ketone. Conn[22] has described the action of hydrolysing the glucoside to HCN as a two step process :

(*a*) The conversion of the glucoside to the corresponding cyanohydrin by a specific β glucosidase (linamarase)

(*b*) Hydrolysis of the cyanohydrin by a hydroxynitrilelyase to give HCN and the corresponding ketone (acetone or propanone).



$R = CH_3,\ C_2H_5$

Linamarase is reported to be deactivated at 72°C.

Various methods of *assay* for the cyanogenic glucosides have been used The assay generally has three phases :

(i) liberation of HCN from the glucoside

(ii) isolation of the cyanide *and*

(iii) quantitation of the cyanide.

Autolysis of the glucoside following maceration, acid hydrolysis, addition of extra enzyme or a combination of these methods yields HCN which is then distilled and estimated after reaction with alkaline picrate, silver nitrate, benzidine derivatives, ferrous sulphate or with other reagents that form coloured compounds with the cyanide.

It appears that most of the early controversy on HCN content of manioc and manioc products, was due to incomplete liberation of HCN, by the methods of assay used.

Most of the early assays [24, 53, 71], depended on either autolysis with endogenous linamarase or on acid hydrolysis with varying strengths of sulphuric acid. Autolysis is not satisfactory as endogenous enzyme levels are low and it therefore requires long incubation times, during which competing reactions appear to lower the total potential yield of the cyanide[80]. Acid hydrolysis has been a popular method inspite of the studies of Wood[99] who warned that amides and ammonia are formed under these conditions, which lowers the amount of HCN released, drastically. In order to test this theory, pure linamarin was hydrolysed with varying normalities of sulphuric acid[48]. The results (*Table I*) showed that :

(*a*) Reduced yields are obtained with acid hydrolysis

(*b*) The normality of acid used affects yield markedly.

A similar conclusion has been reached with studies using plant tissue[80].

Table I. Acid hydrolysis of Linamarin

Treatment	HCN released (%)	Apparent glucoside remaining* (%)
Enzyme hydrolysis	83	
Acid Hydrolysis†		
0.1 N acid	02	81
0.2 N	02	
1 N	08	41
2 N	28	37
4.5 N	47	05
7 N	55	02
9 N	53	02
12 N	51	02
14 N	34	00
18 N	08	00

†Linamarin with the appropriate strength of acid was steam distilled for 1.5 hours.

*The glucoside remaining after acid hydrolysis was determined by adjusting pH to 5·0 followed by hydrolysis by the enzyme. The results are corrected for residual free cyanide from acid treatment (approximately 2%).

Wood[99], de Bruijn[25] and Zitnak[102] have recommended the addition of extra enzyme for assays. This concept has been shown to be correct by recent studies by our group[80], at the CISIR.

The isolation of the cyanide has also produced problems. Air aspiration, a popular method of isolation of HCN formed, has been found wanting[80]. The pH of the medium is important, as very acid pH results in increased binding of HCN[80, 102].

Zitnak[102] has recently outlined the problem arising out of poor sampling techniques which can give misleading results. This problem together with the incorrect assumption that acid treatment results in complete hydrolysis reduces the value of results obtained in many early studies, on cyanogenic glucoside content of manioc.

2.2 **Factors affecting glucoside concentration:** The concentration of cyanogenic glucosides in manioc depends on the variety, stage of development and environment[25]. All parts of the plant contain these compounds. The concentration varies markedly from tissue to tissue, the tubers and the leaves containing the largest amount of glucoside[65]. However, there are variations in glucoside content within the same tissue *e.g.* the young leaves have more toxic factors than the old leaves. The proximal end of the root has more glucoside than the distal end[25].

Soil conditions affect glucoside content markedly *e.g.* while increased nitrogen and drought increased glucoside content, increased potassium and farmyard manure decreased its level. Glucoside content was also found to increase at the onset of rains[25].

Several other factors seemed to affect glucoside concentration : (*a*) shading of plants increased the level in the leaves and reduced the level in the roots ; (*b*) planting of cuttings vertically resulted in less glucoside in the tubers than if the cuttings were planted horizontally ; (*c*) ringing of the stem and leaf elimination resulted in at least a temporary decrease in the toxicity of tubers[25]. Such data are interesting and perhaps with more work the information may be applied practically.

It has also been suggested that growth regulators like NAA, IAA *etc.* may be used to produce tubers with reduced glucoside[46, 88].

2.3 **Elimination of cyanogenic compounds:** Both enzyme and glucoside are found throughout the plant (the enzyme is present in largest quantities in the leaf where it can be found in quantities over 100 fold that of the tubers). However, in fresh tissues the enzyme and substrate are not in contact (intracellular localisation ?). Crushing and soaking in water facilitates contact between the two and hydrogen cyanide is liberated[53]. Most methods of detoxification involve this principle[71]; but it is critical to give the enzyme sufficient time to act[67].

Boiling has been used to eliminate toxic factors. This has been successful in the case of leaves where enzyme concentration is high and diffusion barriers are small[25]. Boiling tissues with low concentration of enzyme while causing elimination of most of the cyanide, probably results in the quick elimination of enzymic activity (which accounts for the remaining glucoside). According to de Bruijn[25], 90 % of the glucoside was still present after boiling. However, earlier studies[23, 78] did not report any glucoside after boiling (poor analysis ?). Several intermediate values have been reported. The amount of glucoside could be predicted to vary widely depending on:

(i) the extent tubers are sliced
(ii) the amount of washing
(iii) enzyme activity
(iv) time taken for the temperature to rise to 70° C.
(v) initial concentration of glucoside, etc.

Adriaens [2] suggested that glucose can detoxify HCN formed but this has been disclaimed by de Bruijn[25].

2.4. **Ingestion of toxic factors:** Toxic factors of manioc include free cyanide, cyanohydrins and glucoside. The toxicity of cyanide is well known. The lethal dose of prussic acid varies from person to person. The average lethal dose for an adult is reported to be between 50 and 60 mg[54] but the amount also appears to be affected by the type of other foods eaten with it[72]. The lethal effect of cyanide is caused by its reaction with the iron in hemoglobin (and possibly cytochromes) and copper in cytochrome oxidase. However, very little free cyanide (less than 5 p.p.m.) is consumed after the normal cooking procedures in this country for manioc : *peel, cut, wash, boil in open vessel, throw out water and reboil.* On the other hand, considerable amounts of glucoside could be ingested especially if the initial glucoside content is high. It might be expected that quantities of glucoside consumed might, in some cases, exceed the toxic level if it were free cyanide. Although the glucoside itself may not be toxic it has to be considered as a potential source of cyanide.

It is possible that the reason why deaths recorded due to manioc consumption are rare, is due to a detoxification mechanism suggested by Clark[19]. His hypothesis is that the sulphoamino acids of proteins react with the cyanogenic glucoside and prevent the liberation of HCN. The hypothesis has been given more credence by his later studies[20], which showed that high cassava diets short in methionine resulted in symptoms of HCN poisoning and by those of Osuntokun *et al*[73] and Montgomery[66] who showed that consumption of manioc as a staple food (2 to 3 meals a day) was associated with a rise of the blood thiocyanate level. However, there seems to be no evidence that it is the glucoside and not free cyanide that is detoxified.

More recently Montgomery[66] outlined the *pathways of metabolism* of inorganic cyanide. At least three pathways appear to exist : (*a*) the conversion of cyanide to thiocyanate by thiosulphate catalysed by the enzyme rhodanase, (*b*) the conversion of cyanide to thiocyanate by reaction with 3 - mercaptopyruvate catalysed by another sulphotransferase. Cysteine and cystine are the precursors of 3 - mercaptopyruvate, (*c*) uptake of cyanide by vitamin B - 12.

The concept that lethal quantities of cyanide could be ingested as glucoside without toxification is significant when one considers the effect said to be caused by some vegetable juices on manioc consumers. It has been commonly reported that if uncooked vegetable foods are taken with manioc, the consumer is subject to violent discomfort that may even result in death[54]. In this country, ginger is popularly believed to cause this effect. It is also believed that some vegetable juices contain an enzyme that will decompose linamarin. It seems equally possible that these vegetable juices may be interfering with a detoxification mechanism.

Recent work by our group[51] have shown that very small amounts of cyanide can be released from linamarin by ginger extracts; the effect is only 10^{-4} to 10^{-5} times that from an equal weight of manioc and is likely to be due to the effect of non-specific β glucosidases. Hence it is difficult to explain the popularly believed effects on this basis. However, it is not possible to rule out adverse effects being caused by eating manioc and ginger together because the mechanism of the effect may not be as what it was hitherto assumed.

It has also been suggested that gut flora can cause the conversion of glucoside to cyanide.[5] However work in this laboratory[51] has shown that coliforms (7 types tested) cannot do this ; work with other bacteria is planned.

Another problem is the popular belief of toxification after the consumption of old and damaged tubers. Although these tubers will contain more free cyanide, the latter will be driven off to a large extent by boiling. These tubers generally have bluish or greyish areas said to be due to the formation of metal cyanides. Some metal cyanides are insoluble and would not be eliminated by standard cooking procedures but would be converted to free cyanide in the stomach. More scientific data are needed to answer three questions in this area :

(i) how relatively toxic are these old tubers ?

(ii) are metal cyanides the only possible cause for the presumed increase in toxicity?

(iii) do cyanohydrins play any role in this connection ?

All these indicate that provided the tubers are fresh, and normal precautions (of eliminating cyanide) are followed, there would be little, if any, danger of loss of life. Worthy of more consideration is the possibility that repeated sub-lethal doses of cyanide (or glucoside) might cause cumulative poisoning.

Sub-lethal doses of cyanide (and/or glucoside) are reported to cause high blood thiocyanate levels which are associated with neurological diseases[73,74]. No doubt some of the diseases attributed to manioc have been due to nutritional imbalance caused by regular consumption of manioc without an adequate supplement of proteins and vitamins. However, Nigerian Ataxis Neuropathy was not relieved by the administration of vitamins, the lack of which was supposed to cause the disease. Although evidence is not complete it is highly suggestive. Extensive manioc consumption (staple food, 2—3 meals a day over a period of years) is also associated with:

(i) Goitre[25,33,34] probably due to higher levels of thiocyanate which is reported to be goitrogenic

(ii) Lebers Optic Atrophy[98] which is reported to be due to hereditary defect in cyanide detoxification *and*

(iii) K Diabetes[76].

It might be pertinent to note that most methods of preparation of manioc products still result in residual glucoside.[53] Also, several studies have shown the presence of glucoside and/or cyanide in manioc chips and flour, in some cases the quantity being high[80],[85] while other studies did not detect toxic factors[8].

The conclusion reached is that several aspects pertaining to the toxic factors in manioc are still unknown. The situation demands extensive research of an interdisciplinary nature.

3. *Products from Manioc*[96]

Two common forms of manioc carbohydrate are available (i) *Starch* and (ii) *Flour*. Starch is the pure carbohydrate while flour, (the ground product of chips) in addition to starch, contains protein, cellulose and other constituents of the tuber cells.

Two types of *starch* can be prepared from manioc : (i) edible starch and (ii) industrial starch. The essential difference between the two are that edible starch needs : (*a*) better raw material and hygiene ; (*b*) equipment made of brass, bronze, aluminium or stainless steel[9] or other equipment which would not form insoluble products with HCN (such as iron or mild steel). Some questions may be raised on the use of some of the above materials suggested by Biema & Shipman[9] ; (*c*) precautions to be taken during the settling and drying process to prevent microbial growth ; (*d*) water which has been treated (e.g. lime-alum) and is bacteriologically satisfactory.

Studies in several laboratories, including the CISIR[49], have shown that a clean manioc starch is not difficult to prepare. However, the big problem is the efficient disintegration of cells ; although manioc contains 28—35% starch, generally only two thirds of this is available due to incomplete disintegration of cells.

Manioc consists mainly of carbohydrate which is highly digestible[12,41]. However, its protein content is very low[61] (around 1%) but the quality of proteins is good. Due to its low *protein* content, manioc consumption in the absence of adequate protein is not advisable. Studies have shown, however, that if an additional protein source is provided, manioc could be used extensively as a staple food (assuming of course, that cyanide has been eliminated). Protein sources that can be used include groundnut[28,68,69] gram flour and fish flour[87], chick pea and coconut soya flour[94]. Yeast protein and skim milk have also been used as a protein supplement.

Processed manioc can be used as a source of calories in the manufacture of *food products* such as (i) bread[13,38], roti[53], stringhoppers[42] macaroni[92] and chappati[91]. Drawbacks to its extensive use in bread are, (*a*) the lack of protein which affects the textural quality of the products and (*b*) possible toxic factors. Considerable work has been done to improve textural quality and nutritional value by blending with other flours and by addition of sources of protein[64,27,57,58,59].

Several minor products have been produced for consumption from manioc, such as gari and farinha (microbiologically fermented products) and tapioca ("sago")[47].

Manioc starch has been used as a starting material for the production of *glucose*[10,30,77,84].

Glucose can be produced by acid hydrolysis or enzymically with the starch hydrolysing enzyme glucoamylase. This enzyme has been produced using local raw materials by our group[50] and it is capable of giving a 90% yield (on theoretical), of glucose.

Manioc has been used as a raw material for production by a *fermentation process* of beer[1], ethanol[6], vegetable cheeses[14,21], yeast[29], glycerol[63] and citric acid[75]. Manioc starch also finds a place as a minor component in several food products.

Manioc has been used extensively in animal and poultry *feed*[3,44,62]. Manioc starch has enormous potential for industrial use. The use of manioc starch in the textile[36], paper[4] and adhesive industries is well known. Manioc glue (NaOH treatment) has had considerable use in wood furniture manufacture[35] before the success of synthetic adhesives.

Manioc starch can be used as a raw material for the manufacture of *modified starches ;* cationic starch[15]; starch esters[16], dialdehyde starch[95], oxidised starch[55,83,101], $AlCl_3$ treated starch[56]; starch ethers[60]; cross-linked starch[100] etc. The wide applicability of modified starches in industry is well recognised[97]. Manioc starch has properties which differ from that of other starches and it is probable that highly specialised modified starches may be prepared from it.

The use of manioc starch in the *adhesive* industry deserves special mention. This source of starch was considered indispensable in the production of remoistening gums[45]. Alkaline oxidized manioc starch[52] and enzyme modified manioc starch[37] have also been extensively used as adhesives.

Manioc has also been used as source of raw material for the production of acetone and butanol[43], cellulose[63], particle board from stalks[39] and HCN for coagulating rubber latex[90] ; manioc leaves have been used as a protein source[32,79,82].

4. *Manioc and Pollution*

The manioc starch industry is a significant source of water pollution in Thailand[93] and Brazil[7]. In Thailand, heavy pollution gives a strong offensive odour and the fauna and flora of waterways into which the effluent is discharged are greatly affected[93]. Pollution can be observed even in the small scale operations in Sri Lanka.

The main waste products in a manioc starch industry are (i) pulp wastes and (ii) wash water. The pulp waste contains large amounts of starch and is quickly susceptible to microbial attack. The wash water has a biological oxygen demand of over 4,000 mg/l and contains about 1% total solids[93].

Further, the water will have a cyanide content (free and bound) probably well over the allowed level for surface disposal.

Although waste treatment is desirable it is often restricted by financial resources. The ideal solution would be the utilization of wastes, (which could offset costs of treatment or possibly even result in a profit). Such studies have been made in Thailand[93]. In this country manioc pulp waste has been made use of as a cattle feed after drying and milling. (*Private communication*). Laboratory trials by our research group[49] have shown that manioc pulp waste can be used as a source of carbohydrate for yeast production. A mixture of acid hydrolysed pulp with added wash water and $(NH_4)_2SO_4$ can produce a 15% yield (on manioc pulp) of yeast cell material. The wash water itself supports yeast growth.

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New Outlook on the Pest Management of Tea

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Abstract An intensive ten year study on the pest ecology of tea has spotlighted some aspects which explain the unsatisfactory chemical control of many pests of tea, such as, tea tortrix, mites and shot-hole borer beetle. Evidence from past experimentation has revealed that in many instances the use of insecticides for pest control in tea has proved to be neither ecologically feasible nor economically worthwhile.

It is suggested that in future pest control in tea should be oriented towards bio-cultural techniques of regulating pest populations.

Introduction

Subsequent to the overdependence on the insecticidal method in pest control in 1960's and the sad consequences of this approach, today we are becoming more and more aware of the importance of an ecological basis to pest control studies. Since then, many have attempted to pay lip service to 'Integrated control', meaning the definite inclusion of the use of insecticides, along with the combination of other methods. But, it is interesting to note that one who may be called the *father* of integrated control has now equated 'integrated control' with "just good entomology" and has advocated the need "to implement, at the practical level for the farmer, an ecologically and economically sound programme of plant protection."[21]

In Sri Lanka, in respect of tea entomology, the evaluation of results of ten years of pest ecological studies, particularly in relation to the most serious pest of tea, (*Camellia sinensis* L), the shot-hole borer beetle, (*Xyleborus fornicatus* Eichh.) has revealed substantial information on the unique aspects of the tea crop and its pest ecology. This paper deals with these unique aspects of the pest ecology of tea and explains the reasons for the unsatisfactory insecticidal control of many pests of tea, such as, shot-hole borer beetle, tea tortrix (*Homona coffearia* Nietner) and mites. Further, it focusses attention on the need to be mindful of the very salient ecological features of the tea crop itself, in relation to the ecology of the tea pests, with the adoption of an ecological approach to the pest management of tea [2, 3], so that control measures will be safer, effective and economical.

Experimental

The ten years of ecological studies on the shot-hole borer beetle pest, from 1962 to 1972, [4, 5, 6], revealed some surprising aspects of this pest. This led to the re-analysis of all insecticidal trials, for the last 15 years, on shot-hole borer beetle, [1, 9, 14] on tortrix [11, 16] and on mites [10, 17, 20] Further, all experimentation reported in the Annual Reports for the years 1956—1970, of the Tea Research Institute of Sri Lanka have been considered and re-evaluated in detail. In all, 148 chemical trials have been re-analysed ; 83 on shot-hole borer beetle, 16 on tea tortrix and 49 on mites.

Results and Discussion

Some unique features of the tea crop

The tea crop has unique characters, which will influence its pest ecology in a very special way. These are :-

1. It is a perennial crop grown in extensive monoculture, in the tropical environment.
2. The harvest is vegetative and is collected at high frequencies, once every 4 days to 10 days, extending over a long period of time, varying from 2 to 4 years or more.
3. The very lengthy period of susceptibility of the crop to tea pests, as much as 2 to 4 years or more, practically the entire length of the pruning cycle.
4. The complete loss of crop for any length of time is unusual ;
5. The harvested crop of green leaf has a low unit value because the 23% out-turn in the manufactured tea fetches an average profit of only 40 to 60 cts/Kg., so that the returns from crop losses recovered do not compensate for the relatively high cost of insecticidal sprays.
6. The pest damage is not known to affect the quality of the final product.
7. The crop environment in tea includes effective natural control agencies of predatory and parasitic insects, pathogenic micro-organisms and

other biotic forms, which should be regarded as an asset in economic pest management and be preserved and developed.

There is considerable experimental evidence from chemical trials on both tortrix and mites, that should no chemical control be attempted, both pests are frequently brought under natural control, within periods of ½ to 3½ months [16, 17]. On the other hand, a classic example of the unleashing of secondary pests, by the use of persistent organochlorine insecticides is provided in tea in Sri Lanka [12], by the outbreaks of caterpillar pests such as tea tortrix, the twig caterpillar, (*Ectropis bhurmitra* Walker) and the looper caterpillar, (*Buzura strigaria* Moore), following the application of dieldrin for shot-hole borer control. Subsequently, the gradual restoration of their natural control, by just discontinuing the use of dieldrin sprays, is valuable evidence of the important part played by natural control of pests by beneficial organisms, both known and unknown[15], in the tea environment. It is also well known that heptachlor application used for shot-hole borer control unleashes the secondary pests of tortrix, twig and looper caterpillars, while DDT that was used to control these caterpillar pests unleashes mite infestations. This is certainly a tragic story of a vicious cycle of pests, brought about by a very injudicious pest control programme. The more important adverse aspect of the above pest control programme is the inevitable deterioration of the natural balance of pest/predator complex in tea, which in the long run, will inevitably lead to increasing costs of pest control. Here it is necessary to focus attention on the fact that in a tropical, perennial, extensive monoculture, such as tea, the adverse effects of the use of insecticides will, in the long run, be more drastic than in temperate crop environments or in tropical annual crops.

8. The rate of vegetative regeneration is extremely high in the tea plant in comparison with other perennial crops. Hence, its remarkable capacity to render a *vegetative harvest* at high frequencies, at intervals of a week or less, makes crop losses insignificant in time.

   This remarkable regenerative capacity in tea, which is unknown in other perennial crops, together with its longevity, renders the tea crop extremely suitable for cultural methods of crop protection, whereby cumulative secondary effects of pest damage, as in the cases of shot-hole borer and termites, can readily be minimized, by utilizing this natural regeneration of the plant.

9. Finally, another important factor that regulates insect populations is the pattern of heavy monsoonal and inter-monsoonal thunderstorm

rains experienced in the tea growing districts. These heavy showers not only by themselves reduce insect numbers, but also affect insect populations by bringing about a quick change in the temperature of the micro-climate. Further, it has been observed that pathogenic diseases of caterpillar pests are heaviest during wet weather conditions.

What does experimental evidence on the chemical control of tea pests reveal ?

The evidence that some serious pests like twig and looper caterpillars are kept under very effective natural control is of extreme significance. Because, in the regulation of these two caterpillar pests, all that was needed was to discontinue the use of the dangerous persistent insecticides, such as dieldrin in tea[15]. Similarly, the withdrawal of heptachlor and DDT sprays will contribute much towards the regulation of tortrix and mite pests, respectively.

In many instances of pest infestations, such as caterpillar pests or mites, the best course of action would be **not** to resort to chemical control. This would help the regulation of pests by means of natural agencies. Resort to the chemical control method will prolong the infestation by merely knocking down the peak infestations, while the chemicals will at the same time affect the natural parasitic/predator complex, thus causing deterioration of the natural balance in the crop environment. In many instances, pest infestations are naturally regulated, with time, even though chemical spraying is not resorted to. This, in combination with the fact that the temporary crop loss recovered by chemical spraying does not in the long run compensate in value for the expenses of chemical sprays alone, renders the spraying of chemicals for the control of pests in tea uneconomic and unrealistic.

The shot-hole borer beetle pest. The pest ecology of shot-hole borer beetle and attempts at its chemical control reveal important aspects of this pest.[4,6] The knowledge of the pest ecology of shot-hole borer beetle and the experience with the very limited levels of control of borer beetle populations and borer beetle damage, using dieldrin and heptachlor, indicated that beneficial chemical control of shot-hole borer beetle, which at the same time will be ecologically feasible, cannot be achieved. The loss of crop in borer beetle infested fields (estimated to be around 20% in the second year of the cycle) and the borer beetle damage of galleries in tea stems are serious enough to warrant control measures, but neither is this loss in yield economically recoverable, nor could the damage in galleries be appreciably prevented by the judicious use of insecticides. This makes it difficult to fix any practical ' economic injury levels ' and it will be even more difficult to fix ' economic threshold levels '. For these reasons an ' integrated programme ', *with the use of chemical control,* for the shot-hole borer beetle pest is unattainable. It is essential in the interest of an 'inter-related pest management system' to co-ordinate the management of the whole pest complex in tea, in an ecologically feasible manner, which in the long run will also be economically sound, by the accent on bio-cultural means of pest regulation alone, and the discontinuance of the chemical control of this pest. This approach will enable an increase of the environmental resistance against all pests and at the same time will also aim at the preservation and development of the most valuable natural pest regulatory means, so abundantly available for most of the tea pests. With our present knowledge of the pest ecology of shot-hole borer beetle, it is evident that we will be compelled to live with this pest and only regulate its population by cultural methods, so as to minimize the damage it causes.

Therefore, the best possible solution to the shot-hole borer problem and the basis for the pest management techniques are as follows :

(*a*) Yield losses caused by shot-hole borer beetle could be remedied by extended pruning cycles alone, because, the yield loss caused by shot-hole borer beetle is chiefly in the second year, of which only around 10% could be recovered by the spraying of permissible levels of persistent chemicals, and in addition these yield losses diminish with time, with the extended pruning cycles. Experimental evidence is available of definite trends in compensatory yield increases, towards the end of three year cycles, in the untreated plots of experiments *ME* 3 (1966) and *ME* 5 (1966).[4]

(*b*) Experimental evidence in collaboration with the analysis of field data, in the Mid-Country, reveal that spraying of chemicals for shot-hole borer beetle is uneconomical.[4] It will be more economical **not** to spray for shot-hole borer beetle, *either* in a three year pruning cycle *or* in a four year pruning cycle.

(*c*) Therefore, rather than spraying for shot-hole borer beetle it will be advisable to extend the pruning cycle, to a fourth year in the Mid-Country and to a third year in the Low-Country. Analyses of field data, of the Mid-Country, show that a four year pruning cycle is as good as a three year cycle, yield wise, where the average annual yields are about the same. This practice will bring better economic returns and is agriculturally advantageous.

(*d*) The cultural method of pruning will remedy the more serious secondary damage caused by wood-rot. Wood-rot in tea takes about 10 years to develop into a serious condition. It is *partly* a secondary effect of borer beetle galleries and this could be remedied by the cultural method of selective clean pruning, into wet weather, with the retention of lungs, at each prune.

The tea tortrix pest. The tea environment consists of a wealth of natural parasites and pathogens of the tortrix pest[13]. However, frequently in localized areas, there are temporary infestations of the tortrix pest, which are brought under natural control in ½ to 3½ months time. So much so that it has been a problem to assess the effectiveness of chemical sprays on this caterpillar pest, because the experimental areas soon lose their infestations. It is extremely interesting that to commence and continue the chemical trials on the tortrix pest, the experimental areas had to be sprayed with dieldrin (an insecticide that was prescribed for shot-hole borer beetle control) to induce the pest.[16] The only experiment where an attempt was made to assess the yield loss caused by tortrix infestation (*E 70* — 1968, that was initiated as a mite experiment) was continued for a period of only 22 months. To enable assessment of the effect of loss of crop by the tortrix infestation, the treated plots during this period received 13 rounds of DDT for tortrix control and the ' untreated control plots ' received 3 rounds of dieldrin to induce the tortrix pest. At the end of the experiment, that ran for only 22 months, a yield increase of only 247 Kg/ha (220 lb. made tea/a) or 14% was obtained. It is interesting to note a definite trend in compensatory yield increase, towards the end of a three year cycle, in the infestation induced plots. This experiment provided valuable insight into the chemical control of the tortrix pest, with the use of insecticides and indicated the uneconomic nature of this exercise. There are many more recorded instances of the failure to keep chemical trials of tortrix infestations continuing for sufficient lengths of time, in order to make any precise assessments[22], on account of the natural regulatory agents.

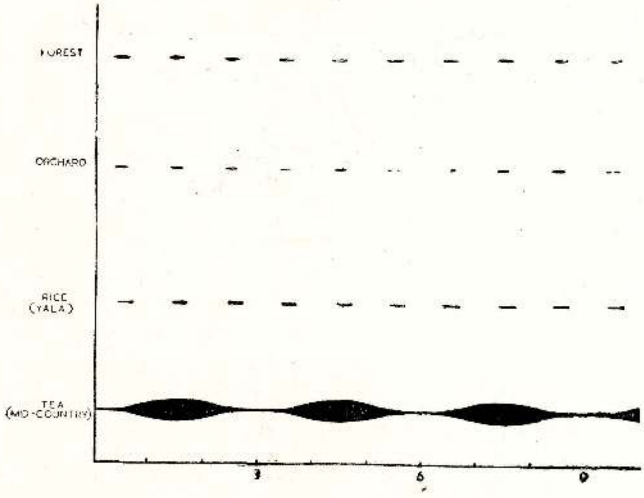
Mite pests. The results of all chemical trials on mites follow a somewhat similar picture to that of the attempts to control tortrix infestations with chemicals. In the case of mites too the experiments could not in many cases be continued because the untreated control plots lost their infestations. There are also several experiments that had to be discontinued on account of the deterioration of the mite infestation to very low numbers. Cranham[7], at the end of a three year period found no significant loss of crop due to mite attack. Again, Cranham[8] reported that in Uva, in the Gonamotawa mite trial, at the end of only 11 months the yield loss recovered was only 10%, in spite of the inducement of mites in ' untreated ' control plots by the spraying of DDT (an insecticide prescribed for the tortrix pest control). Further, experiment *E* 49 (1967) on mites did not show any significant yield loss recovered at the end of the trial, although there were beneficial trends in yield up to the 22nd month. Experiment *E* 50 (1967) too gave similar results. Again, in experiment *E* 21 (1965), in Welimada, Uva district, which ran for a period of four years, where 21 rounds of Kelthane were used to control the mites in the treated plots and 21 rounds of DDT were given to the 'untreated control' plots, to induce mites, the yield loss recovered was only 202 Kg/ha (180 lb. made tea/a) or 3% in spite of the achievement of over 90% population control[17]. Here again, it is interesting to note a definite trend in compensatory yield increase, towards the end of the four year cycle, in the infestation induced plots.

The significance of experimental evidence

The above experimental evidence, viewed as a whole, reveal important aspects of the chemical control of tea pests and their benefits. The most important of these is that although a temporary recovery of yield loss could be obtained by the knock-down effects of chemical sprays, in the long run, at the end of the pruning cycle, of 3 to 4 years or more, the yield loss recovered is either nil or meagre and uneconomic. Another important aspect is that the benefits achieved by the use of insecticides are further reduced by the side-effects they cause. Further, the evidence indicates, that even should no chemical treatment be administered, in many instances of tortrix and mite pests, a natural decline of the pest populations does occur.

The explanation for the above should be sought in the unique aspects of the tea crop, as well as its pest ecology. The lengthy period of the susceptibility of the tea crop explains the difficulty of achieving the profitable protection sought by the use of insecticides (*Figure* 1). The pest populations in tea

FIGURE 1. **Patterns of crop susceptibility***



*A diagramatic representation of the periods of crop susceptibility in tea compared with that of a tropical annual crop such as rice (Yala) and orchard and forest crops in the N. Temperate zone.

TABLE I — **Aspects to be investigated before the recommendation of a pesticide**

Aspects needing investigation	*Normal considerations*	*More important points often overlooked*
1. Evidence of control	*A significant* population kill	1. What *quantum* of damage reduced ? 2. Trends in several well distributed experiments are more important than significant results in one or two experiments
2. Taint) Residues) Health aspects)	These obtain only a cursory attention	These should be fully investigated
3. Side-effects	Evidence of slight side-effects ignored - A pesticide recommended provisionelly - From then on the provisional recommendation is not reviewed in the face of accumulating evidence	1. A slight indication of side-effect, should be sufficient to warrant the removal of a pesticidal recommendation. 2. Pesticidal recommendations must be regularly reviewed.
4. Results of spraying	1. *Protection* primary consideration. 2. *Production* given secondary and cursory attention	1. *Protection* important only if it affects production in the long run. 2. *Production* should be the primary aim, not *protection.*
5. The economics of spraying in relation to yield	Not sufficiently considered	1. Yield ' increases ' from experimental plots should be related to averages for fields on the commercial basis. 2. Yield ' increases ' in short periods in pruning cycle should be related to average yields of pruning cycles. 3. The yield ' increases ' must further be related to yield averages in the various districts, involving various lengths in pruning cycles. 4. Unit costs should be related to costs on large commercial estate scale ; where small yield increases will add to lowering the C.O.P. and thus turning a marginal gain in unit costs, to considerable profit on a commercial scale.
6. Practical considerations	Very little consideration given	The final results obtained from small carefully conducted experimental plots, should accommodate the economics of operations on larger commercial scale, with regard to (1) Dosage of chemical ; (2) Profit margins expected ; (3) Feasibility of recommendations.

also reach their normal peaks and decline, as is the general rule in agricultural pest populations. By the use of pesticides, what is achieved is the immediate knock-down of a part of the population peaks and in many instances this is followed by a prolonged infestation, sometimes on account of the effect of the insecticides on the beneficial natural parasitic insect populations. Therefore, an attempt has been made in *Table* 1 to illustrate the various aspects that should be investigated before the recommendation of a pesticide for any particular pest of tea.

In the past, in tea entomology, there was the habit of indiscriminate attribution of the cause of deaths of tea plant to various pests. One such instance is that the attack of shot-hole borer beetle on young clonal tea is a contributory cause of the death of the clonal plants during severe droughts. It is now known that the death of clonal plants, during a severe drought is chiefly determined by the factors of the type of clone and soil, and it is already shown that there is no relationship between clonal susceptibility to shot-hole borer beetle attack and drought casualties[6]. Similarly, we should be wary of the view that the defoliation of tea bushes consequent of mite attack, is the primary cause of death of these tea bushes in a severe drought, unless this view has the backing of experimental evidence.

Another interesting feature is that dry weather encourages pest infestations and at the same time aggravates the adverse effects of the pest infestations on the crop, at a time where the crop yields are already low, on account of the drought. This creates the false impression that the main cause of the crop loss is the pest infestation and not the drought conditions, thereby exaggerating the actual loss of crop on account of the pest alone. It is true that insect attacks cause loss of crop on account of the drain on the nutrients and/or moisture stress. This is not greatly felt in wet weather, but in dry weather, any loss of nutrient and particularly water stress is more adversely felt by the plant which will tend to show appreciable amount of *temporary* loss of crop. But, no sooner the rains come the plants show quick vegetative regeneration and compensation in yield losses. Therefore, pest infestations during droughts in tea receive a false and undue importance.

The limitations of the insecticidal method of pest control in tea

When all the experimental evidence over the last fifteen years is viewed from the new orientation of the pest ecology of tea, it appears that the routine application of insecticides for many of the tea pests is uneconomical and untenable for the following reasons:

(*a*) The cost of spraying, even one round of a chemical, is more than the anticipated returns from the crop loss recovered, over the entire pruning cycle. This is all the more so, on account of the increasing costs of production and decreasing profit margins, in combination with the increasing costs of spraying chemicals.

(*b*) The apparently large loss in crop is short-termed and in time this loss is compensated with renewed vegetative growth so that at the end of the pruning cycle there is very little or no loss of crop.

(*c*) The evidence that in time the pest attack is controlled under the influence of natural agents, as in the case of tortrix and mites, **or** is diminished to uneconomic injury levels, like in the case of shot-hole borer beetle, as the pruning cycle advances.

(*d*) The chemical spraying causes the deterioration of the environment, by weakening the natural pest regulatory agents. It also leads to the increasing dependence on the use of more chemicals, making the whole operation still more uneconomic.

What should therefore be the new outlook on pest management in tea ?

Pest control in tea, which in the past has been over-dependent on the insecticidal method, with the emphasis on mere protection, should in the future be oriented towards bio-cultural techniques of regulating pest populations, directed primarily on economic production, so that we arrive at safe, effective and economic means of pest management in tea. The procedures that can be adopted are :—

(1) The immediate ban on the use of chlorinated hydrocarbon insecticides, such as DDT, heptachlor and dieldrin, in pest control in tea. This is already done for tea in N. India, by the Tocklai Experimental Station. Gasser[19] advocated this as the primary step necessary for any programme of ‘integrated control’.

(2) The practice of the application of prophylactic sprays should be discontinued. Prophylactic sprays were prescribed for the prevention of the tortrix pest, following dieldrin applications, and still continue to be done for mites.

(3) Any pesticide should be recommended only after a thorough investigation, in all its aspects (*Table* 1), and particularly only if its benefits, both from the point of protection and economics, could justify the cost of the sprays and any adverse effects it causes to the crop environment. It is suggested that the routine application of pesticides be discontinued and replaced by supervised directed spray pro-

grammes, only in cases of large scale outbreaks of insect pests or in very specific instances.

(4) There is the need for more dependence on the bio-cultural methods, which to-date have not been fully investigated. Therefore the necessity arises to develop the environmental resistance against pests and at the same time, to encourage the successful colonization of beneficial biotic populations. Another important approach is the diversification of the environment which could be adopted by the planting of trees, as shade where necessary or as wind belts or even as boundaries. The growth of suitable cover crops is an added means of creating a favourable environment for the development of the beneficial insect populations, because, thereby we provide insect parasites, particularly of the useful hymenopterous insects, with sources of food and shelter.

(5) Finally, there is the need for intensive long term ecological research on the other pests of tea, similar to that being carried out on the shot-hole borer beetle of tea. Research needs to be directed towards cultural means of pest regulation and methods at improving the environment.

Bio-cultural methods of pest control in tea

The immediate, short-term results of insecticidal applications, in killing insect populations, irrespective of the quantum of protection and economic benefits accrued towards increased production, have led many to believe that pest management cannot be achieved without the use of insecticides. This is also true of pest management in tea.

The above attitude to pest control overlooks the more important aspect of the natural environmental resistance to pests, brought about by predatory insects and organisms within the crop environment. It ignores the fact of the slow development, over the years, of natural beneficial insect populations. On the other hand, it also ignores their very gradual deterioration and the consequent imbalance of pest populations which are largely dependent on the agro-cultural methods we adopt down the years. The most hazardous of these agro-cultural methods is the use of non-selective persistent pesticides. In tea, we have good examples of introduced biologically useful parasitic insects taking control of pest populations. One such instance is the natural control of the tortrix caterpillar pest by the introduced *Macrocentrus* parasite.[18] But, what is equally important to realise is the less known or unknown insect/parasite complex in tea that is responsible in keeping several likely pests under check, such as twig and looper caterpillars and perhaps many other unknown pests.

Some attempts to control shot-hole borer beetle by cultural means have failed in the past because these have been applied without a sufficient knowledge of the ecology of the pest. Therefore, those who appear to have little faith in the bio-cultural methods, through an ecological approach, exaggerate the limitations of this approach and assume that in many instances we cannot free ourselves from the dependence on insecticides in tea, while they are unaware of the serious limitations of the gainful use of insecticides in tea.

The cultural method of removing damaged parts, of the tea plant that rapidly regenerates, by suitable pruning which induces new vegetative growth, can be readily applied to minimize damage caused by pests, such as shot-hole borer beetle or termites, that takes many years to develop into serious proportions. Therefore, on account of the highly regenerative capacity and longevity of the tea plant and the very slow build-up of pest damage, the cultural method of pest regulation by pruning takes advantage of the time lag in the development of the pest damage.

Similarly, a ready method of regulating the tortrix pest is the introduction of *Macrocentrus* parasite. However, since 'central breeding' of the parasite for large scale distribution will be a costly process it is suggested that cocoons of the parasite could be merely collected from neighbouring estates, where the attack is nearly under control, at its late stages, and introduced into other fields with new infestations of the pest. This simple method will only require the co-operation of the planters, in any particular district, in sharing the information as regards tortrix infestations and as from where the cocoons of the parasite could be collected at any particular time. The Tea Research Institute could act as a co-ordinating agency to assist planters in obtaining the cocoons of the parasites at the right time. This would be a better means of regulating the tortrix pest than the ineffective and dangerous practice of routine insecticidal applications and should merit trial.

As for mite pests, on the evidence provided above, it would be seen that prophylactic sprays and even routine sprays will not be of much benefit. Again, it is suggested that chronic and repeatedly mite attacked fields could be treated at pruning time with a good spray of any sulphur based pesticide, which would have other advantages on the crop too. Of course, this is only meant as a suggestion worthy of trial, experimentally.

It is therefore significant that we look at pest management in tea, from a long term standpoint, with the background knowledge of the ecology of the crop and pest-ecology of each pest problem individually as well as collectively. This calls for an ecological approach to tea entomology, with increased economic production as the main goal.

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Factors affecting Quality, Strength and Colour of Black Tea Liquors

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Abstract Total and vanillin—reacting polyphenols, total amino-acids, caffeine and polyphenoloxidase activity were higher in young than in mature leaves. The tender stem contained a high level of amino-acids, especially theanine and glutamine, but polyphenoloxidase activity, and contents of polyphenols and caffeine, were relatively low.

Black teas manufactured from leaves of differing maturity, and from tender stems, showed marked differences in organoleptic properties, and in theaflavin and thearubigin contents. The reasons for the differences in quality, strength and colour are ascribed to the proportions of different polyphenols, polyphenoloxidase activity, caffeine, theanine, theogallin and compound G 36 contents of the raw material, from which the black tea was processed.

1. *Introduction*

Black tea of the best quality is made from carefully plucked "flush", comprising the apical bud and two adjacent leaves, (termed first and second leaves), with included stem. There is a marked reduction in quality of the black tea when the raw material used for processing includes the more mature leaves of the tea shoot, but the reasons for this loss of quality are not precisely known. The purpose of this study is to evaluate the nature of the chemical compounds responsible for the differences in quality, strength and colour of the brews derived from black teas processed from tender stem and leaves of differing maturity.

Earlier studies of polyphenols in Assam tea[1] and in Malawi tea[4] grown at Cambridge, England, had shown that the levels of catechin gallates, gallic acid, theogallin and compound G 36 (unidentified, but found to yield gallic acid on hydrolysis) were maximal in the flush and decreased as the leaf matured. On the other hand, the levels of myricetin glycosides and epigallocatechin were markedly higher in mature leaves, whilst derivatives of the flavones, apigenin and luteolin, were detected in mature leaves only. The young stem contained catechins, gallocatechins and gallic acid, together with myricetin glycoside and rhamnoglucoside, and two other unidentified flavanols; compound G 36 was absent from the stem, but chlorogenic and p-coumarylquinic acids were more concentrated in the stem than in the leaves.

In the present investigation on Ceylon tea, the stem, apical bud, first, second, third and fifth leaves were analysed quantitatively for total and vanillin - reacting polyphenols, and semi-quantitatively for some of the individual polyphenols. Analyses were also made of total amino-acids, caffeine, soluble reducing sugars and sucrose, and polyphenoloxidase activity. In addition, the different tissues were processed to black tea, and these black teas were assessed organoleptically, and analysed for theaflavins and thearubigins.

2. *Results and Discussion*

2.1 Polyphenols occur at maximal concentration in the first leaf, and show a steep fall as the leaf matures, and the amount of polyphenols in the stem is relatively low. (*Figure* 1) Semi-quantitative studies, made by inspection of 2-dimensional chromatograms sprayed with diazotized p-nitroaniline, gave results in agreement with those of previous workers[1,4] The steep decrease in polyphenols with leaf age was observed to be due to the progressively lower contents of flavanol gallates in the more mature leaves; the relatively higher level of flavanols in these leaves may be due to reduced esterification[4] or to the effect of a tannase, which causes hydrolysis of the gallates originally present. Flavanol glycosides were more abundant in mature leaves than in young leaves, and although the stem contained relatively low levels of theogallin and other polyphenols, the proportions of p-coumarylquinic and chlorogenic acids were high. The high proportions of these depsides would not, however, be shown in Figure 1, as neither of the reagents used (Folin Ciocalteau and vanillin), forms coloured products with these polyphenolic compounds.

2.2 Amino-acids There was a progressive decrease in amino acid concentration as the leaf matured, but the tender stem contained a high level of amino-acids (*Figure* 2) Paper chromatographic analyses revealed that the high levels of amino-acids in the apical bud and tender stem were due to the abundance of glutamine and theanine in these tissues. The first and second leaves contained relatively less theanine than the third and fifth leaves, but the higher level of total amino-acids in the younger leaves was due mainly to their higher contents of α- alanine and glutamic acid.

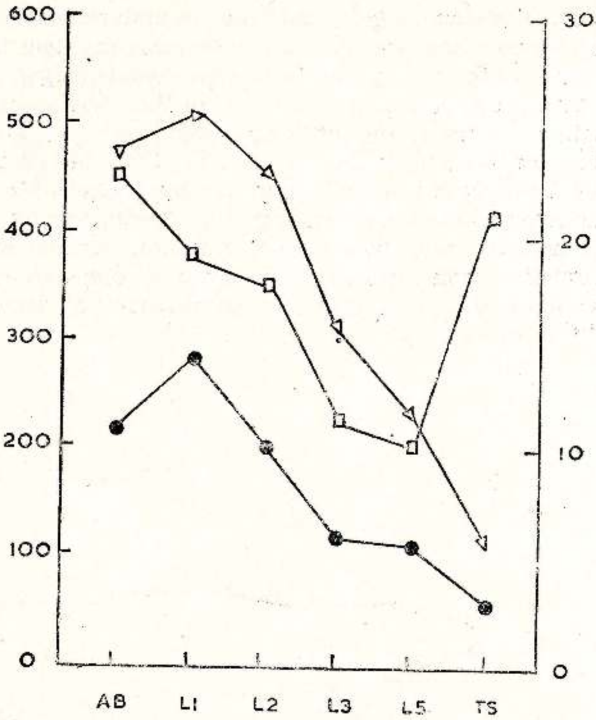
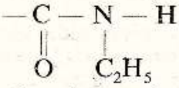


Figure 1. Polyphenol and amino-acid contents of apical bud, leaves of different age, and tender stem of tea shoot.—Δ—Δ, total polyphenols;—●—●, vanillin—reacting polyphenols ;—□—□, total amino-acids ; AB—apical bud; L_1, L_2, L_3, L_5—first, second, third, and fifth leaves respectively; TS—tender stem.

Autoradiographic studies[16] have shown that theanine is biosynthesized in the roots of the plant, and translocated therefrom to the growing shoot tip. The relative amounts of theanine in the different leaves suggest that translocation ceases after a certain level of theanine is attained in the leaf, and that this accumulated theanine is not translocated out of the leaf after it attains maturity. The exact role of theanine in the tea plant has not been clearly defined, but it is possible that this most abundant amino-acid of tea may have a function in protecting the enzymes of the tea leaf from inactivation by polyphenolic and other compounds or elements. Thus, the  $-\underset{\underset{O}{\|}}{C}-\underset{\underset{C_2H_5}{|}}{N}-H$ group in theanine could form hydrogen bonds with polyphenols and may, in this way, prevent the formation of insoluble enzyme-polyphenol complexes, having an activity less than that of the free enzyme. The hydrogen bond formed between phenols and N-substituted amides has been described as one of the strongest types of hydrogen bond[3].

2.3. Caffeine The percentage of caffeine in mature leaves was less than that in young leaves, and was lowest in the tender stem (*Figure* 2). It is possible that caffeine, together with polyphenols, sugars, and amino-acids (other than theanine), may be some of the substances translocated from the mature leaves to the growing shoot tip[12]. A possible role for caffeine in the tea plant may be similar to that of theanine, *viz.* the prevention of insolubilization of enzymes by polyphenols. Evidence in favour of this hypothesis is the finding[5] that caffeine splits the insoluble complexes formed between tannin and enzymes, with no loss in activity of the regenerated enzyme, and the observation (*unpublished*) that polyphenols were dissociated from insoluble gelatine-tea polyphenol complexes by the addition of caffeine.

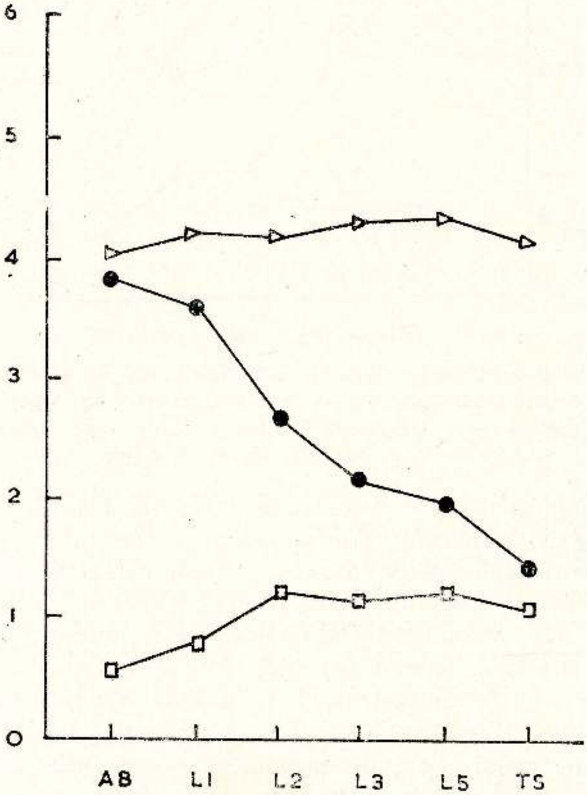


Figure 2. Caffeine and soluble sugar contents of apical bud, leaves of different age and tender stem of tea shoot.—●—● caffeine;—Δ—Δ,sucrose;— □ — □ , reducing sugars. AB, L_1, L_2, L_3, L_5, TS, as in Figure 1.

2.4. Soluble carbohydrates There were only small variations in the amounts of soluble sugars and sucrose in young and in mature leaves, although the amounts were higher in the latter (*Figure* 2). This higher level is maintained in spite of the possible translocation of sugars from these leaves to the young leaves, and suggests that the mature leaves are more actively photosynthetic than the younger leaves. Translocation of photosynthetically formed material from mature leaves to the growing apex of tea plants has been demonstrated earlier.[12]

2.5. Polyphenoloxidase activity was found to decrease as the leaf matured, and to be comparatively low in the tender stem (*Figure* 3). The high enzyme activity in young leaves is in accord with the finding[17] that polyphenoloxidase, localized in the leaf epidermal cells, was of equal activity in both the upper and lower epidermis in young leaves, but

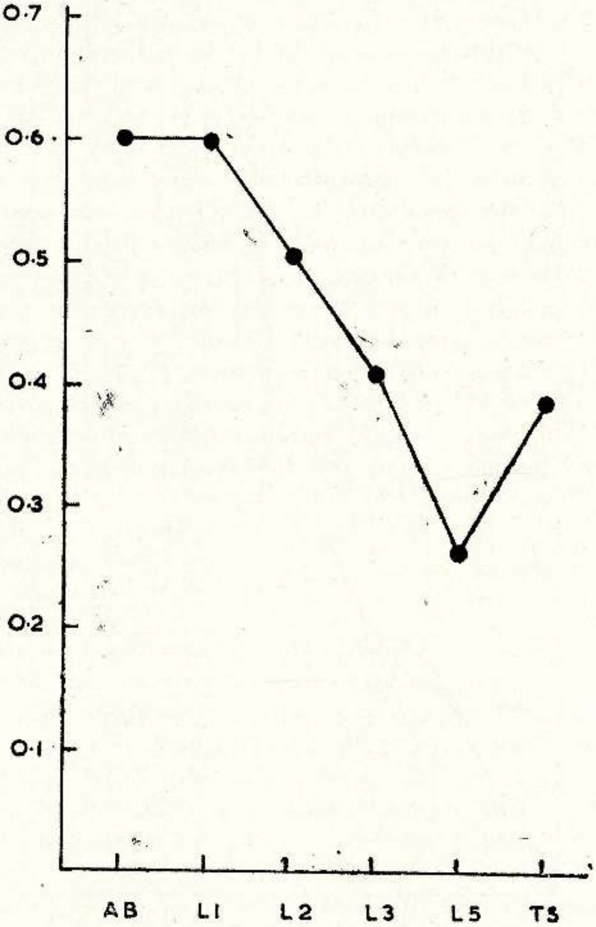


Figure 3. Production of material absorbing at 380 mμ (theaflavins) by enzyme extracts of apical bud, leaves of different age, and tender stem. (Polyphenoloxidase activity). AB, L_1, L_2, L_3, TS, as in Figure 1.

decreased in activity, first in the upper, and then in the lower epidermis as well, as the leaf matured. If one of the roles of polyphenoloxidase and polyphenols in young tea leaves is to produce quinones and other compounds, which confer protection against invading pathogens and pests,[17,19] it would seem that this mode of protection loses its importance as the leaves mature and become resistant, as a result of morphological and chemical changes. The occurrence of such changes is indicated by the increased content of ethanol-insoluble material in mature, as compared to immature leaves.[13]

2.6 Theaflavin formation is highest in the tender stem and lowest in the fifth leaf (*Figure* 4). The latter observation is to be expected in view of the low polyphenol content and low polyphenoloxidase activity of the fifth leaf. The finding that the tender stem surpasses the apical bud in theaflavin formation is unexpected, because the apical bud contained a level of polyphenols and enzyme activity which were higher than that of the stem. The apparently anomalous result may be explained by assuming that the stem contains the *optimal* levels[18] and proportions of the different tea polyphenols and enzyme, which are necessary for the rapid formation of theaflavins, and that this reaction goes to completion due to the low levels or absence of compounds which interfere with or inhibit the enzyme oxidation reaction. *e. g.* intermediates in the formation of theaflavins, and other inhibitory polyphenols - one of which may be compound G 36. It is also possible that the high level of theanine in the stem contributes towards protecting the polyphenoloxidase from inactivation or insolubilization by the oxidation and polymerization

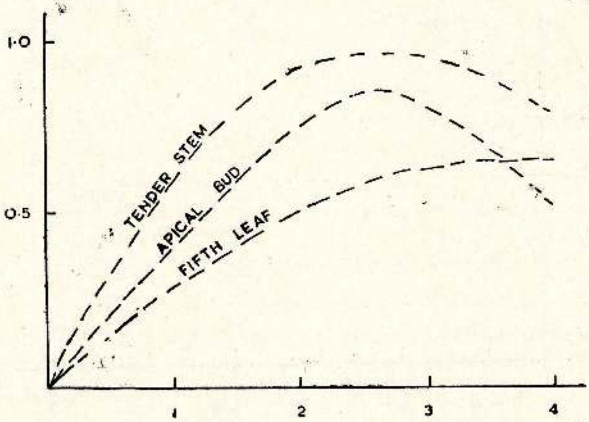


Figure 4. Theaflavin formation by tender stem, apical bud, and fifth leaf. Material macerated and extracts for analysis made in boiling water after different periods of fermentation.

products, and it is noteworthy that the rate of theaflavin formation does not fall steeply after reaching a maximum, as in the case of the apical bud.

2.7. **Black teas manufactured from the different tissues** showed marked differences in organoleptic characteristics (*Figure* 5), as assessed by professional tea tasters, and in theaflavin and thearubigin content (*Figure* 6).

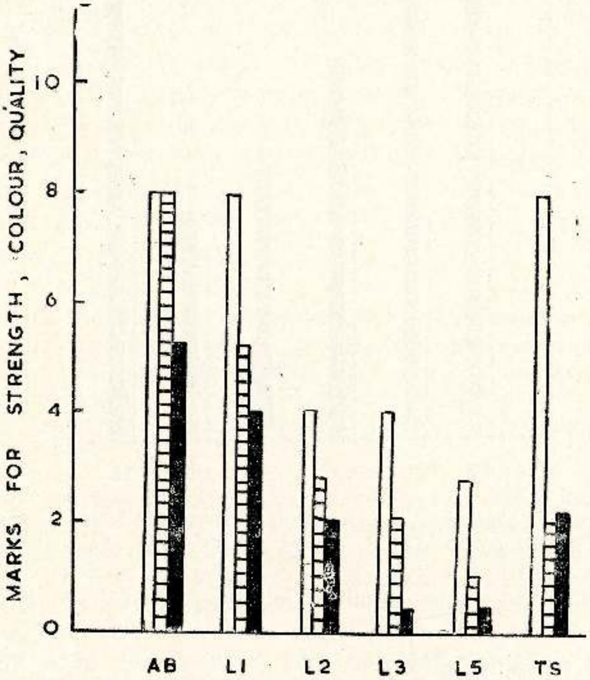


Figure 5. Organoleptic properties of processed black teas. ■, quality ; ▤, strength ; □ , colour. AB, L_1, L_2, L_3, L_5, TS as in Figure 1.

The tea tasters' scores for quality, colour and strength showed a progressive decrease for the teas made from the apical bud to the fifth leaf, and this is in accord with the chemical composition and polyphenoloxidase activity of the various tissues. The processed stems showed the highest level of theaflavin, and had a fair score for quality which was, however, less than that for the processed apical buds, suggesting that theaflavin content *per se* is not responsible for quality. Furthermore, the processed stem also contained the highest thearubigin content of all the black teas, but colour and strength were less than that of the teas from apical bud or from the first leaf. The reasons for these findings may be the comparatively low level of theogallin, and lack of compound G 36, in the stem; theogallin has been reported[2] to be one of the factors affecting the cash

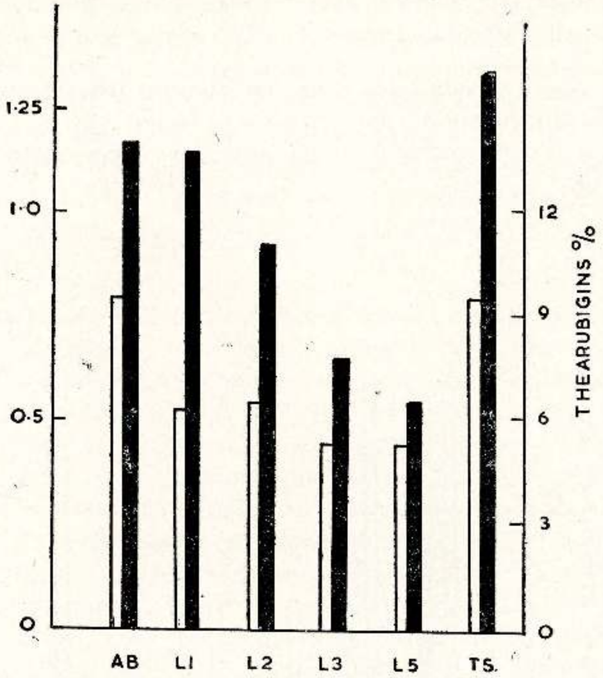


Figure 6. Theaflavin and thearubigin contents of processed black tea. ■, theaflavins ; ☐ , thearubigins ; AB, L_1, L_2, L_3, L_5, TS, as in Figure 1.

valuation, briskness and quality of black teas. It is also possible that the high level of theanine in the stem may prevent the organoleptic perception of strength. Theanine has been found to be incorporated as part of the thearubigin complex[8] and a high level of theanine may result in an alteration of the relative proportions of the constituents of this complex, and so alter its contribution to strength and colour.

3. *Conclusions*

The high quality of black teas made from the apical bud and young leaves may be regarded as being due to the high level of polyphenols and polyphenoloxidase activity, which results in the oxidation of polyphenols to compounds responsible for quality. The oxidation reaction is favoured by the high levels of theanine and caffeine, which protect the enzyme from insolubilization by the oxidation products. Black teas made from tender stems were also of high quality in spite of the low polyphenol content and low polyphenoloxidase activity in this plant material, and it is suggested that quality was here due to the correct balance of polyphenols and enzyme, protection of the enzyme by the comparatively high level of theanine, adequate caffeine, and the absence of compound G 36. In mature leaves the low polyphenol content together with low polyphenoloxidase activity and low caffeine content resulted in the production of teas of low quality.

The contents of theaflavins and thearubigins were high in black teas derived from the apical bud and stem, and both were rated high for quality. Apical bud black teas were rated high for colour and strength as well, but stem black teas were scored much lower for these characteristics, suggesting that the thearubigins of these two teas were different, possibly due to the relatively high theanine, low theogallin and absence of compound G 36 in the stem.

4. *Experimental*

4.1 **Plant material**. Shoots were obtained from tea bushes (*Camellia sinensis* L. var. *assamica*, clone TRI 2023), which had been allowed to grow freely for three weeks after being plucked. Five hundred shoots were separated into apical bud, first, second, third and fifth leaves, and tender stem included by the bud, first and second leaves. A portion of each material was extracted for analyses of chemical constituents, and the remainder processed to black tea by conventional methods.

4.2 **Extracts** for polyphenols, amino-acids, and soluble sugars were made by macerating the material (10g) for 5 min. with 80% (v/v) aqueous ethanol (50 ml), and boiling the suspension obtained for 5 min. The suspension was filtered through glass wool, and the residue re-extracted for 10 min. periods with boiling 80% (v/v) aqueous ethanol (twice, 50 ml portions) Extracts were pooled and made up to 100 ml.

4.3 **Polyphenols.** Total and vanillin—reacting polyphenols were estimated colorimetrically, according to the methods of Swain and Hillis[15] using Folin — Ciocalteau and vanillin reagents.

4.4 **Amino-acids** were estimated with ninhydrin, according to Yemm and Cocking[20].

4.5 **Caffeine** was estimated by the A.O.A.C. method, described by Pearson[7].

4.6 **Soluble reducing sugars and sucrose.** The polyphenols were first removed by Polyclar AT[11]. Reducing sugars were determined colorimetrically, using Somogyi[14] and Nelson[6] reagents. Sucrose was determined after hydrolysis with invertase (β-D- fructofuranoside fructohydrolase 3.2.1.2.6.). Invertase (0.01 ml BDH suspension stabilized with glycerol) was added to 1.0 ml. solution containing sucrose (20-80 mg), and the hydrolysis allowed to proceed for 1 hour at 27°C. The reducing sugars were then estimated as above.

4.7 **Theaflavins and thearubigins** were determined spectrophotometrically, as described by Roberts and Smith[9].

4.8 **Polyphenoloxidase extraction** was carried out in a cold room at 4° C by grinding the tissue (20 g), frozen at −20° C, Polyclar AT (10 g), acid-washed sand (4g) and

0.5 M phosphate buffer, pH 5.8 (60 ml)[10], The homogenate was filtered through muslin, and Polyclar AT (1 g) was added to the filtrate to remove any residual polyphenols, and the suspension re-filtered. The filtrate was centrifuged at (3000 r.p.m. for 10 min. at —5° C.) The supernatant, which still contained traces of chlorogenic and p-coumarylquinic acids, was used as the enzyme extract.

4.9 **Polyphenoloxidase activity** was determined spectrophotometrically by measuring the amount of material absorbing at 380 mμ ; enzyme extract (0.5 ml). (equivalent t othe enzyme present in 1 g. of fresh tissue) was incubated (20 min. at 37° C), with a mixture of epigallocatechin gallate and epicatechin gallate (18 mg.) dissolved in 0.5 M phosphate buffer, pH 5.8 (2,5 ml). The mixture of gal lates was obtained by elution of the appropriate spots from 2-dimensional chromatograms, developed first in butanol—acetic acid—water (6: 1: 2 v/v) and then in acetic acid (6% v/v), and estimated with reference to a standard curve prepared by using varying concentrations of D-catechin.

4.10. **Polyphenols and amino-acids.** Semi-quantitative assessments of polyphenols were made by visual inspection of 2—dimensional chromatograms developed in butanol—acetic acid—water (6:1:2, v/v) and acetic acid (6%, v/v), and sprayed with diazotized p-nitroaniline. For amino-acids the chromatograms were developed first in the same butanol—acetic acid—water solvent, and then in phenol (500 g. in 125 ml. water), followed by spraying with ninhydrin (0.4% w/v) in ethanol, (95% v/v) containing $CoCl_2.6H_2O$ (0.2% w/v)

4.11. **Processing of black teas.** The various tea shoot entities (250 g) were withered for 18 hours, macerated in miniature tea rollers, and allowed to ferment for 2½ hr. Fermentation was arrested by 'firing' for 21 min. at 80°C and the black teas stored for 2 weeks before organoleptic assessment and analyses for theaflavins and thearubigins, were made.

Acknowledgement

We gratefully acknowledge the assistance of Mr. Ranjit Sri Nissanka and Mr. G. Balasingham in assessing the organoleptic properties of the processed teas.

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Chemistry of the Guttiferae of Ceylon*

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Abstract : The structure elucidation of the compounds isolated from the bark and timber extractives of different species of the family Guttiferae belonging to the genera, *Calophyllum* L., *Kayea* Wall, *Mesua* L., and *Garcinia* L., is discussed. The account includes the chemistry of xanthones, coumarins, bark acids, biflavanoids and triterpenes.

In Ceylon there are over 3300 flowering plant species belonging to 1294 genera and 192 families. Of these about 830 plant species belonging to 342 genera and 94 families are endemic to Ceylon. Our programme on the chemical investigation of the endemic plants envisages the study of the extractives obtained from the bark, timber and flowers or fruits with different solvents and isolation of pure compounds and chemically identifying them. Besides it is planned to carry out pharmacological studies on the total extracts and pure constituents.

Figure 1 shows the distribution of the endemic species in Ceylon.

The present review will be confined to the results we have obtained on the species of the family Guttiferae found in Ceylon. The family Guttiferae and its tribes[24] are given in *Chart A*.

There is difference of opinion about the inclusion of the genus *Hypericum* L. in the family Guttiferae (Clausiaceae). The family derives its name from *gutta* or gamboge, a yellow resinous juice that can be tapped from *Garcinia hanburyi* (Cambodia, Siam). Gamboge first reached Europe at the beginning of the 17th century. It is used medicinally as a laxative and provides a bright yellow pigment. *Garcinia mangostana* L., the mangostin tree, provides one of the most highly prized edible fruits of the tropics. Another tropical delicacy, the mamey

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*Based on the *Kandiah Memorial Lecture*, of the Institute of Chemistry, Ceylon, delivered on July 14, 1972.

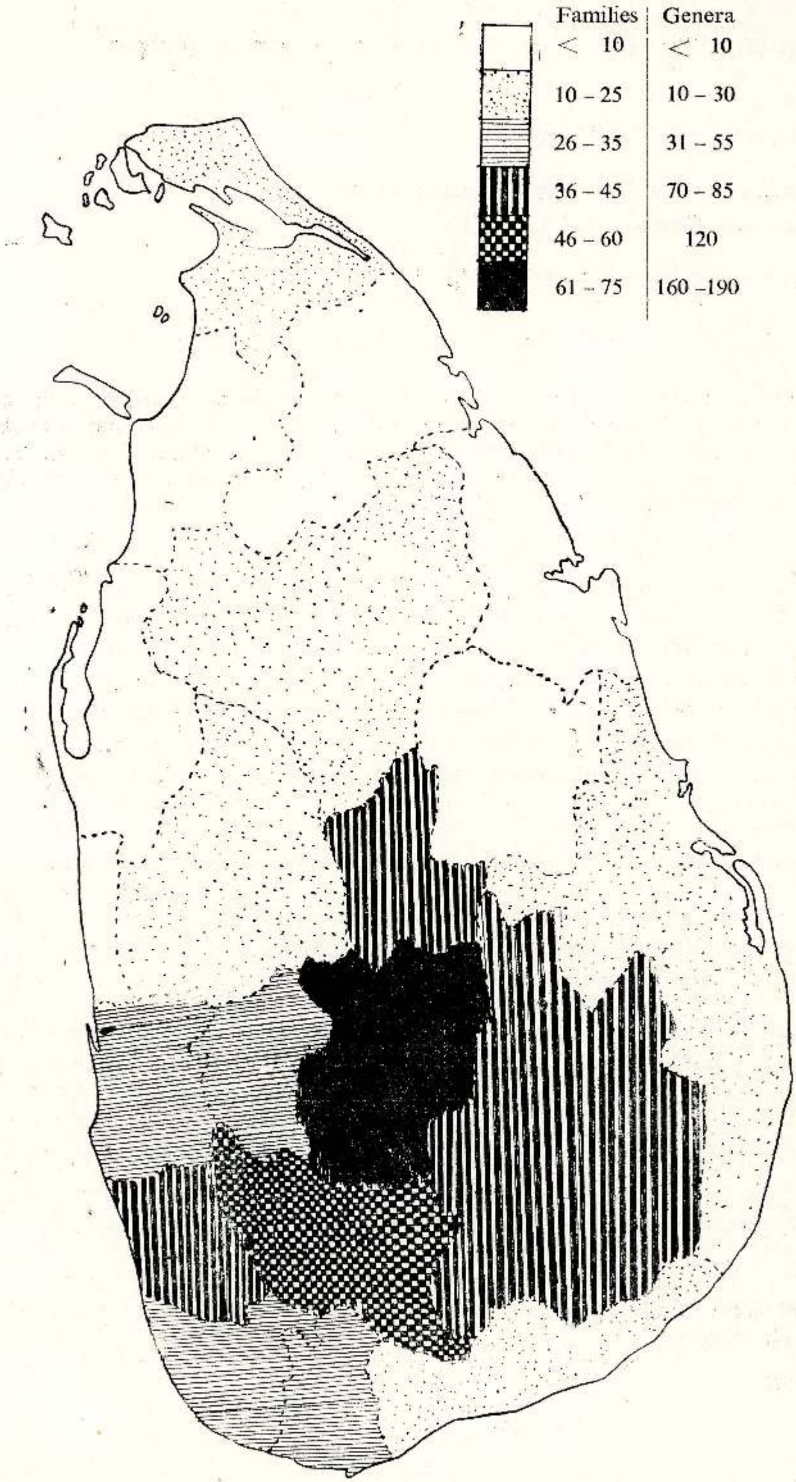

Figure 1. Distribution of the endemic species in Ceylon

Chart A Guttiferae

Subfamily	*Tribe*	*Genera*
1. Kielmeyeroideae	Kielmeyereae Caraipeae	*Kielmeyera* Mart., *Marila* Sw., *Caraipa*
2. Calophylloideae	Calophylleae	*Mesua* L., *Mammea* L., *Calophyllum* L., *Kayea* Wall,
	Endodesmieae	*Endodesmia* Benth.
3. Clusioideae	Clusieae	*Clusia* L., *Chrysoclamys* Poepp, *Tovimita* Auble
	Garcinieae	*Garcinia* L., *Pentaphalangium* Warb., *Allanblackia* Oliv., *Rheedia* L.,
4. Moronobeoideae		*Pentadesma* Sabine, *Moronobea* Auble, *Montrouziera* Planch, *Platonia* Mart., *Symphonia* L.
5. Lorostermonoideae		*Lorostemon* Duck
6. Hypericoideae	Vismieae	*Vismia* Vand, *Psorospermum* Spach, *Haronga* Thou.
	Cratoxyleae Hypericeae	*Cratoxylon* Blume *Ascyrum* Vand. *Hypericum* L.

or St. Domingo apricot, is the fruit of *Mammea americana*, a large tree of the West Indies and now widely cultivated in many tropical countries. The seeds of the butter tree or tallow tree of tropical Africa, *Pentadesma butyracea* Sabine are the source of an edible fat used for cooking, called Lamy or Kanga or Sierra Leone Butter and used for soap, margarine and candles. In Ceylon all parts of the tree and seeds of *C.inophyllum* L. (*S* — domba)[4] have medicinal uses and medicinal uses have also been reported from *C.apetalum* Willd (whole plant), *C.tomentosum* Wight (seeds), *Garcinia indica* Chois (bark oil, fruits), *Garcinia mangostana* L. (pericarp), *Garcinia echinocarpa* Thw. (seed oil) etc. and several other *Calophyllum* L. species (known generally in *Sinhala* as " Kina ") are used for timber purposes.

Most plants belonging to this family are trees and are generally confined to the warm humid tropics. Some of the genera and species of this family have been found to be endemic to certain land masses e.g. *Kielmeyera* is confined to Brazil and other South American countries and *Symphonia* is confined to Africa and to the Malagasy Republic.[16] It is possible that all these plants originated from a common stock that existed before the continental drift and after separation of the continents those plants became confined to different land masses where they underwent different generic changes to give rise to different species. The spread of these species was checked by various barriers such as oceans, large mountains and large patches of dryland and hence these new species became confined to land masses only. In Ceylon there are about 22 plants belonging to the subfamilies Calophylloideae and Clusioideae (***Chart B***).

Chart B GUTTIFERAE SPECIES IN CEYLON

HYPERICUM L.		*H. mysurense* Wight & Arn	
		H. japonicum Thunb	
		H. humifusum L.	
MESUA L.		*M. ferrea* L.	
		M. ferrea L. (form *M.salicina* Pl. & Tr.)	Ia
	*	*M. thwaitesii* Planch & Triana	I
CALOPHYLLUM L.		*C. soulattri* Burm.f.	
	*	*C. calaba* L.	II
		C. pulcherrimum Wall ex Choisy	III
		C. inophyllum L.	IV
		C. tomentosum Wight	
	*	*C. bracteatum* Thw.	V
	*	*C. thwaitesii* Planch & Triana	VI
		C. trapezifolium Thw.	VII
	*	*C. cuneifolium* Thw.	VIII
	*	*C. cordato-oblongum* Thw.	IX
	*	*C. walkeri* Wight	X
KAYEA Wall	*	*K. stylosa* Thw.	XI
CLUSIA L.		*C. rosea* Jacq	XII
GARCINIA L.		*G. cambogia* (Gaertn) Desr.	
		G. morella Desr.	
		G. echinocarpa Thw.	XIII
	*	*G. terpnophylla* Thw.	XIV
		G. spicata (Wight & Arn) Hook.f.	
		G. tinctoria (DC) W. F. Wight	
		G. mangostana L.	

* Endemic species

We have investigated all but one of the endemic species which we have not been able to locate yet.†

Over 57 Guttiferae species[7] have been investigated in the literature, without the *Hypericum* species, *Kielmeyera* (9 species) *Calophyllum* (20 species), and *Garcinia* (16 species) being the major ones. We have investigated 9 *Calophyllum*, 3 *Garcinia*, 2 *Mesua*, 1 *Kayea* and 1 *Clusia* species.

Xanthones

Over 80 different xanthones[7] have been isolated from the different species. Xanthones with isopentenyl and geranyl substituents are common in the family

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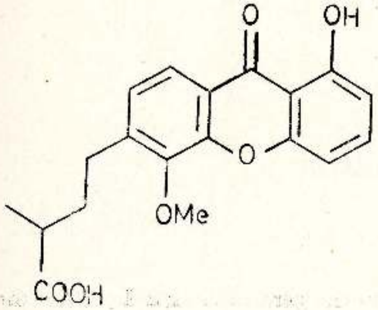
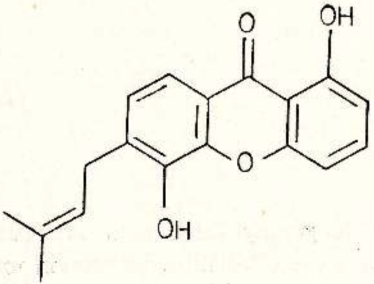
† Professor A. Kosterman has provided this species recently and it is being investigated.

Guttiferae. Scheinmann[7] and others have suggested that substituents other than oxygen may have chemotaxonomic significance. One or two isopentenyl side chains may be present eg. Scriblitifolic acid (1), guanandin (2) and mangostin (3)



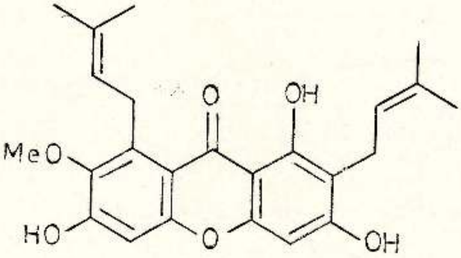
SCRIBLITIFOLIC ACID

1



GUANANDIN

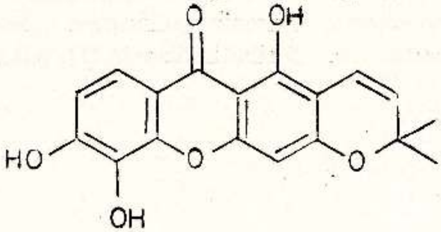
2



MANGOSTIN

3

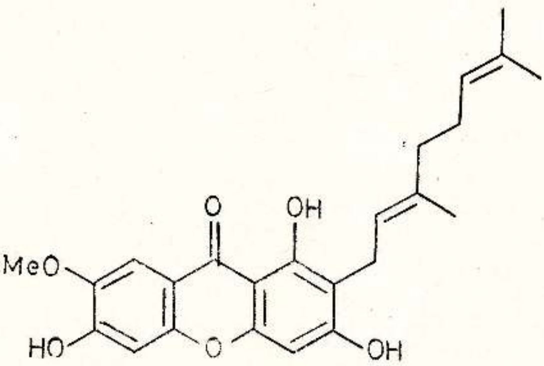
In some cases one or two of these isopentenyl substituents are modified by terminal cyclisation on to the *o*-hydroxy group to give a chromene system as in the case of jacareubin (4)



JACAREUBIN

4

The geranyl substituents are found mostly in the genus *Garcinia* L. In some cases these substituents remain unmodified as for example cowaxanthone (5) and cowanin (6) but in others the substituents undergo cyclisation as in the case of gambogic acid (7a) and morellin (7b).

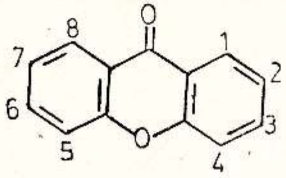


COWAXANTHONE

5

The different xanthones isolated from the various species can be classified according to their oxygenation pattern. Of these we have isolated 22 different xanthones of which seven are new xanthones and some are being reported for the first time in certain genera. The structures of the known xanthones and the frequency of their isolation are listed in *Table* 1.

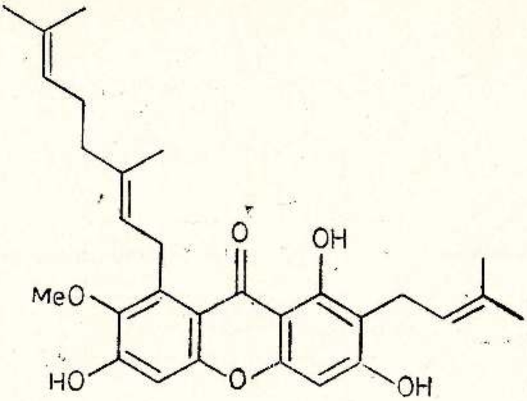
Table 1



Xanthone	**Plant isolated from in Ceylon***
Mono oxygenated.	
2-hydroxyxanthone 4-hydroxyxanthone 2-methoxyxanthone	I(a)
Di-oxygenated	
1, 5-dihydroxyxanthone	I, Ia, V, VI, VII, X, XIII, XIV (*from* 6 *other species by others*)
1, 5-dihydroxy-6 (3′-methylbut-2-enyl) xanthone (guanandin)	II, VIII (*from* 3 *species by others*)
1-hydroxy-5-methoxy-6 (3′-carboxylbutyl) xanthone (scriblitifolic acid 1)	II, VIII (*from* 1 *species by others*)
1, 7-dihydroxyxanthone	I, Ia, II, V, VI, VII, VIII, X, XIV (*from* 12 *species by others*)
Tri-oxygenated	
1, 3-dimethoxy-5-hydroxyxanthone	I (*from* 1 *species by others*)
1,3,5-trihydroxy-2-(3-methyl-but-2-enyl) xanthone	VIII (*from* 1 *species by others*)
6-deoxyjacareubin	II, V, VIII (*from* 7 *species by others*)
1,6-dihydroxy-5-methoxyxanthone (buchanoxanthone)	II, VIII (*from* 4 *species by others*)
1, 5, 6-trihydroxyxanthone	I, I(a), II (*from* 8 *species by others*)
2, 8-dihydroxy-1-methoxyxanthone	II (*from* 1 *species by others*)
Tetraoxygenated	
Jacareubin (4)	II, V, VI, VII, VIII, X (*from* 8 *species by others*)
Mangostin (3)	XIV (*from* 1 *species by others*)

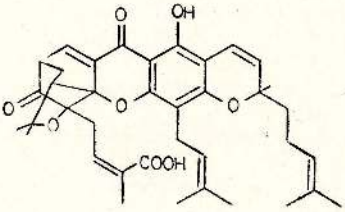
* Roman numerals refer to names of Guttiferae species given in *Chart B*

A brief outline of the methods used in fixing the structures of the new xanthones follows. From the barks of the *Calophyllum* species we have isolated for the first time three new di-isoprenylated xanthones, calabaxanthone, thwaitesixanthone and trapezifolixanthone. Of these calabaxanthone,[31] (9) isolated from *C. calaba* L., showed in its uv, a resemblance to osajaxanthone(8).



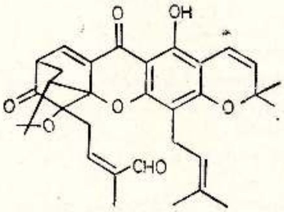
COWANIN

6



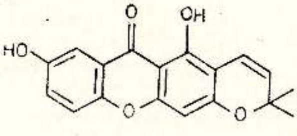
GAMBOGIC ACID

7a



MORELLIN

7b



OSAJAXANTHONE

8

This was further confirmed by comparison of the uv spectrum of tetrahydrocalabaxanthone with dihydro-osajaxanthone (*Table* 2).

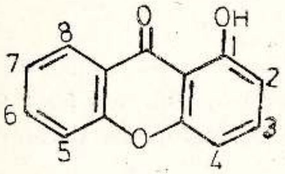
Table 2. Comparison of u.v. absorption maxima, nm (ε x 10^{-3})

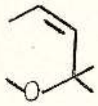
							Solvent
Osajaxanthone	238 (19.0)	249 (18.0)	286 (47.10)		339 (8.2)	382 (4.8)	*a*
Osajaxanthone mono methyl ether	240 (21.4)	248 (19.9)	286 (43.6)		339 (9.0)	375 (4.5)	*a*
Calabaxanthone	240 (34.01)		287 (77.25)	292sh (72.21)	314 (28.23)	384 (8.42)	*b*
Tetrahydrocalaba-xanthone	241 (63.36)	262 (64.84)			316 (36.43)	369 (9.11)	*b*
Dihydro-osajaxanthone	237 (30.9)	262 (33.48)			316 (16.22)	380 (6.25)	*a*
Dihydro-osajaxanthone monomethyl ether	233 (31.62)	259 (39.81)			316 (16.59)	372 (6.16)	*a*
1, 3-dihydroxy-7-methoxyxanthone	235 (28.28)	259 (32.36)			311 (13.8)	369 (6.31)	*a*
1-hydroxy-3, 7-dimeth oxyxanthone	231 (34.71)	259 (38.55)			292 (24.36)	369 (6.78)	*a*
1-hydroxy-3, 7-dimeth oxy-2-(1, 1-dimethyl allyl)-xanthone	233 (34.63)	264 (37.18)			311 (18.32)	372 (6.84)	*a*
2-(3,3-dimethylallyl)-1,3,7-trihydroxy-xanthone	241 (33.87)	263 (32.89)			314 (17.14)	337 (6.50)	*a*

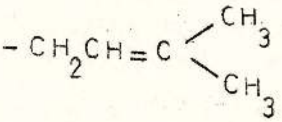
Solvent a- ethanol, *b-* methanol

The NMR spectrum gave information for the presence of

(*a*) dihydro pyran ring
(*b*) isopentenyl group
(*c*) OMe group

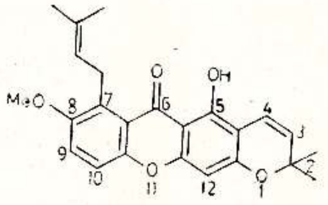





$-CH_2CH=C\begin{matrix}CH_3\\CH_3\end{matrix}$

$-OCH_3$

The three aromatic protons appeared as two singlets at τ 2.73 (2H) and τ 3.77 (1H). The high chemical shift of the signal at τ 3.77 (1H) indicated that this proton should be located in the electron rich phloroglucinol ring of calabaxanthone. As the compound gave a positive Gibbs test, a proton para to xanthone C-1 position should be free. Since there is only a single proton in the phloroglucinol nucleus which is assigned to xanthone C-4 position the 2,2-dimethyl-2*H*-pyrano ring must be attached to the 2,3-position of the xanthone ring giving a linear arrangement of the 2*H*- pyrano ring with respect to the rest of the molecule



CALABAXANTHONE

9

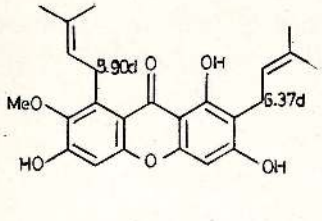
Further confirmation of this linear arrangement of the 2,2-dimethyl-2*H*-pyrano ring was obtained from the proton shifts observed on its acetate (*Table* 3)

Table 3. Chemical shift differences

	C_4 -H	C_3 -H	C_{12} -H
Calabaxanthone	3.25	4.43	3.77
Calabaxanthone acetate	3.46	4.24	3.31
Diamagnetic ($\Delta\tau$)	+ 0.21		
Paramagnetic ($\Delta\tau$)		—0.19	—0.44

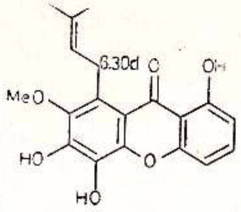
A positive diamagnetic shift and a negative paramagnetic shift for a linear 2*H* - pyrano ring proton and negative paramagnetic shift for the C_{12} aromatic proton has been recorded for similar systems[3].

The position of the isopentenyl group was fixed by comparison of the -CH_2- NMR signals of the isopentenyl group with the same group in mangostin (3) and celibixanthone (10).



MANGOSTIN

3

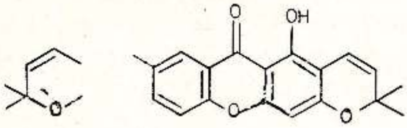


CELIBIXANTHONE

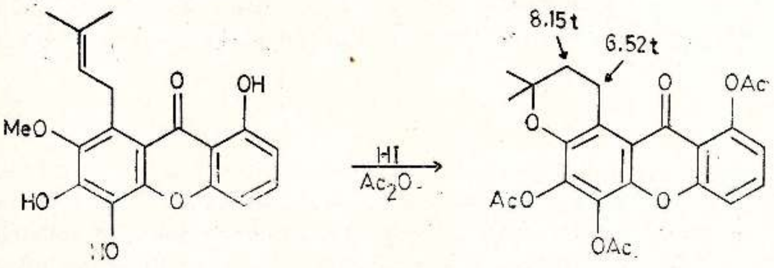
10

On the above basis the structure of calabaxanthone becomes 2,2-dimethyl-5-hydroxy-8-methoxy-7- (3-methylbut-2-enyl)-2*H*,6*H*-pyrano- (3,2-b) xanthen--6-one (9).

For the structure of thwaitesixanthone isolated from *C. thwaitesii* Planch & Triana we need only consider the establishment of the two dihydro pyran rings. Similar evidence as for calabaxanthone gives the partial structure :

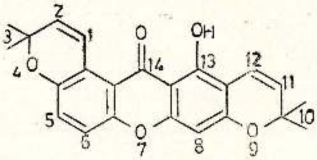


The other 2,2-dimethyl-2*H*-pyrano ring could be attached in two ways to give a linear or an angular molecule. Evidence which supported an angular fusion was (*a*) the low τ 1.98 (1H) for one of the doublets in thwaitesixanthone and its methyl ether (*b*) the NMR spectrum of tetrahydrothwaitesixanthone contained three sets of triplets at τ 6.50(2H), τ 7.28(2H) and τ 8.18(4H) Two of the three values are similar to those of tetrahydrocalabaxanthone τ 7.28(2H) and τ 8.18(2H). The other pair was similar to those found for ring closed celibixanthone (11) as shown below :



10 11

On this basis thwaitesixanthone was formulated as 13-hydroxy-3, 10-tetramethyl -3*H*, 10*H*, 14*H*-dipyrano [3,2,*a* :2′, 3′-*i*]-xanthen-14-one (12)



THWAITESIXANTHONE

12

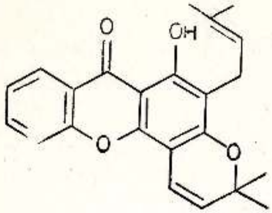
The above structure was confirmed by studying the proton shifts on the acetate, of thwaitesixanthone. (*Table* 4)

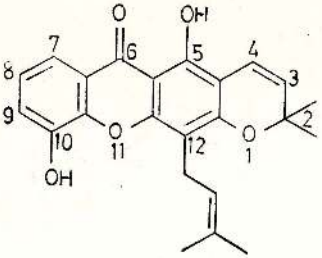
Table 4. Chemical shift differences

	C_{12} –H	C_{11} –H	C_8 –H	C_1 –H	C_2 –H
Thwaitesixanthone	3.26	4.41	3.72	1.98	4.19
Thwaitesixanthone acetate	3.50	4.28	3.33	2.04	4.23
Diamagnetic ($\Delta\tau$)	+0.24			+0.06	+0.05
Paramagnetic ($\Delta\tau$)		—0.13	—0.39		
	linear		*angular*		

A positive diamagnetic shift and a negative paramagnetic shift for a linear 2,2-dimethyl-2*H*-pyrano ring proton and a negative paramagnetic shift for C-8 aromatic proton is in agreement with the linear ring. The positive shift for the other ring protons may be indicative of the angular placement of the other pyrano ring.

Trapezifolixanthone (13) isolated from *C. trapezifolium* Thw. on similar arguments would be formulated in two ways by the 2,2-dimethyl-2*H*-pyran-ring being fused at C_2 or C_4. If it was fused at C_4 as in structure (14)



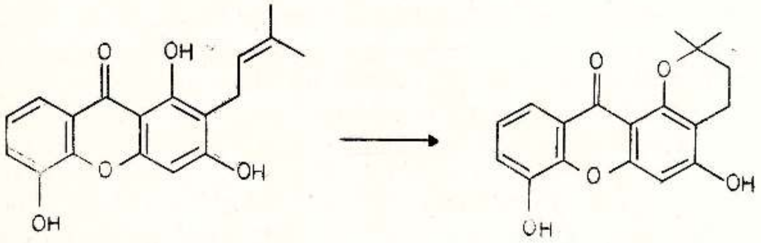


TRAPEZIFOLIXANTHONE

13 14

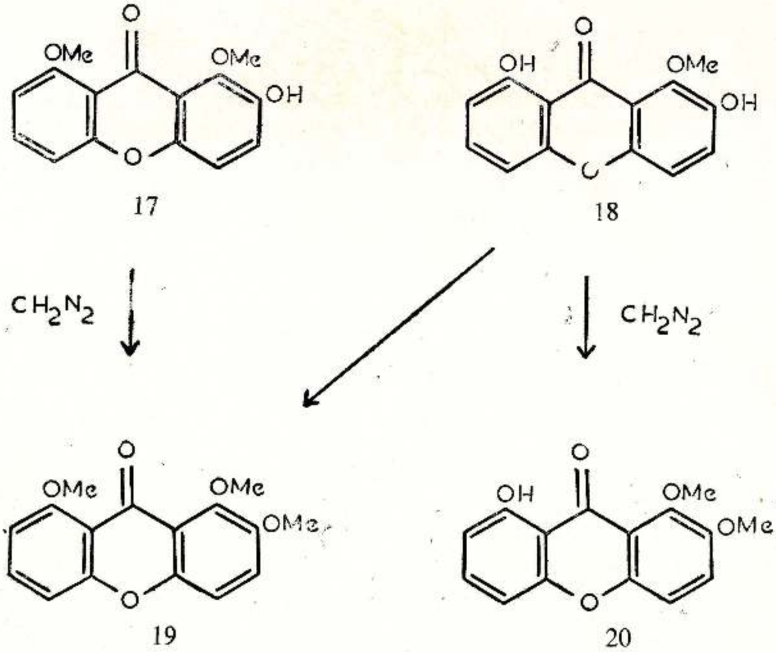
the methylbut-2-enyl side chain should be located at C_2 and the compound would ring close to form a dihydropyran type of compound on boiling with formic acid. However, trapezifolixanthone failed to cyclise under the conditions and hence it was concluded that it had 2,2-dimethyl-2*H*-pyrano ring fused to C_2 with 2-methylbut-2-enyl group in position C_4. Its structure therefore, becomes 5, 10-dihydroxy- 2,2-dimethyl-12- (3-methylbut-2-enyl)-2*H*, 6*H*-pyrano -[3,2-*b*] xanthene-6-one (13). This structure has been confirmed by synthesis by Professor Jain (*personal communication*) at the University of Jammu, India.

This method has been used by Selliah[29] to establish the structure of 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (15) by cyclising it to (16) with formic acid at room temperature.



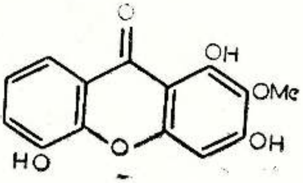
15 16

The chemical method used can be illustrated with 2-hydroxy- 1,8-dimethoxyxanthone[31] (17). Thus methylation of compound (17) with diazomethane and methylation of 2,8-dihydroxy-1-methoxyxanthone (18) with dimethyl sulphate converted both into 1,2,8-trimethoxyxanthone (19).



This suggested that compound (17) was a 1,2,8-trioxygenated xanthone. The NMR spectrum indicated the presence of two methoxy groups at τ 6.22 (3H) two *AB* doublets at τ 2.68 (1H) and τ 2.80(1H) (J_{AB} 8.4 Hz), two doublets at τ 3.08 (1H) and τ 2.98 (1H) (J_{AB} 8.4 Hz), and a triplet at τ 2.3 (1H J 9 Hz). This suggested that there were two ortho protons in one ring and three vicinal protons in the other. The fact that the compound was fully methylated with diazomethane suggested that the hydroxy group was free and not chelated to the carbonyl group. This observation was further confirmed by the u.v. spectrum which was unaltered by the addition of alcoholic aluminium chloride. 8-hydroxy-1, 2-methoxyxanthone (20) obtained from 2,8-dihydroxy-1-methoxy xanthone (18) by methylation of diazomethane was found to be different from compound (17) which must, therefore, be 2-hydroxy-1, 8-dimethoxyxanthone.

The structure of the tetraoxygenated compound from *C. bracteatum* Thw. was assigned as the u.v. spectrum showed characteristic xanthone peaks and i.r. spectrum had strong bands at 3275 cm^{-1} (free OH) and 1652 cm^{-1} (chelated C = O). The NMR spectrum indicated the presence of one methoxy group and four nuclear protons. On methylation with diazomethane a trimethoxy compound and with dimethyl sulphate a tetramethoxy compound were formed indicating the presence of three free hydroxy groups and one of which is chelated, and therefore at C-1. This was further supported by the bathochromic shift of maxima in the uv spectrum on addition of aluminium chloride. Since the compound was stable to alkali, C-2 and C-4 are not substituted. Furthermore, the original u.v. spectrum was unaltered by the addition of NaOAc-H_3BO_3 which ruled out the possibility of ortho-hydroxy groups. The presence of hydroxy group at C-3 *or* C-6 allows a xanthone to be ionized by sodium acetate, and the original uv spectrum of the compound (21) was altered in the longer wavelength region on the addition of sodium acetate which indicated the presence of a hydroxy group at C-3, -5 or -6.



21

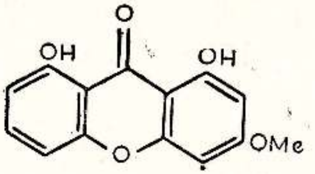
The NMR spectrum of 1-hydroxy-2, 3, 5-trimethoxy xanthone, obtained by the methylation of compound (21) with diazomethane showed resonances at τ 3.49 (4H,*s*), τ 2.78 (6- and 7-H, *m*) and τ 2.29 (8-H, *q*) and these values are very similar to that of the signals of the compound (21). The tetramethyl ether of (21) was shown to be identical with 1, 2, 3, 5-tetramethoxyxanthone by comparison with an authentic sample (mixed mp and NMR). This established the oxygenation pattern of the compound as that of a 1, 2, 3, 5-tetraoxygenated system.

One hydroxy group has already been assigned to C-1 and since there was no shift of the uv absorption with NaOAc—H_3BO_3 the methoxy group was assigned to C-2. On biosynthetic evidence, a phloroglucinol oxygenation pattern is required for the xanthone nucleus and hence the second hydroxy group is likely to be at C-3. Based on the above observations the structure of the new xanthone was deduced as 1, 3, 5- trihydroxy-2-methoxyxanthone[31] (21).

The structure of the pentaoxygenated compound from *C. bracteatum* Thw. was assigned as follows:

The uv and ir spectra indicated that it was a xanthone. The NMR spectrum showed signals at τ -3.2(1H) and τ -0.3(1H) for two hydroxy-groups and at τ 6.07 (3H,*s*), τ 6.15 (3H, *s*) and τ 6.25(3H,*s*) for three methoxy groups. The aromatic protons appeared as two doublets at τ 2.55 (1H) and τ 2.85 (1H) (J 9 Hz) and a sharp singlet at τ 3.37(1H). The di-acetate had a similar NMR spectrum for the aromatic protons and the three methoxy groups and the acetyl signals were observed at τ 7.49 (3H) and τ 7.66 (3H). The coupling pattern shown in the NMR spectra of the parent compound and its di-acetate suggested the presence of two aromatic protons in one ring and a single proton in the other. The high values of the aromatic proton signals in the NMR indicated that C-1 and C-8 of the xanthone nucleus were substituted.

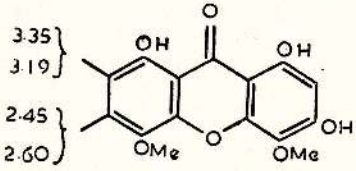
The addition of aluminium chloride produced a bathochromic shift of the maxima in the uv spectrum, thereby locating one of the hydroxy groups at C—1. The addition of NaOAc and NaOAc—H_3 BO_3 did not effect the uv spectrum. The compound was also stable to alkali. Therefore the second hydroxy group could not be at C-2, -4 or -5. The negative values observed for the two hydroxy groups in the NMR spectrum suggested that they are probably located at C-1 and C-8, as has been reported by Markham[26] for 1,8-dihydroxyxanthones. This is confirmed by the absence of signals in the NMR characteristic of nuclear protons at C-1, or C-8. On biosynthetic evidence, one aromatic ring in the xanthone nucleus should have a phloroglucinol oxygenation pattern. Therefore one oxygen function a methoxy group, is likely to be located at C-3. Hence the partial structure for the compound could be formulated as B:



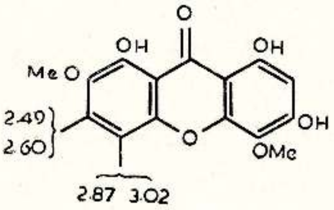
B

OMe at C-2 or -4
OMe at C-5 or -7

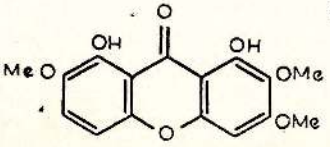
The other two methoxy groups have to be assigned to C-7 or -5 and C-2 or -4. The NMR signal of H-2 always appears at somewhat higher fields than that of H-4, for a given set of hydroxy and methoxy substituents. The exact values depend on the type of substituent in each ring but in general H-2 appears at τ 3.5 - 3.7 and H-4 at τ 3.2 - 3.5. On this basis the singlet at τ 3.37 was assigned to H-4 and the methoxy group at C-2. By comparison of the τ values observed for H-5 and -6 and H-6 and -7 in compounds (22a) and (22b), the methoxy group was assigned to C-7. Based on the above arguments, the structure is 1,8-dihydroxy-2,3,7-trimethoxyxanthone (23)



22a



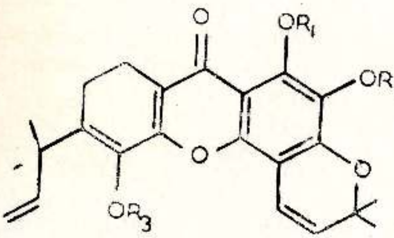
22b



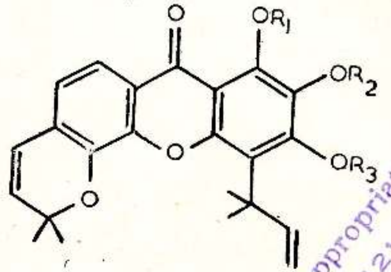
23

Kayeaxanthone[29] obtained from the bark of *Kayea stylosa* Thw. showed the u.v. maxima at 260^{sh} (log ε 4.29), 269 (4.31), 292 (4.63) and 368 (3.42) nm and strong ir absorption at 1644 (chelated carbonyl) and 3250 cm^{-1} in the IR These spectra suggested that the pigment was axanthone.

The NMR spectrum showed the presence of a 1,1-dimethyl-prop-2-enyl side chain, as in the case of symphoxanthone and globuxanthone, the olefinic protons appearing as an ABX system at τ 3.51, 5.01, and 5.15. The signals of the gem dimethyl appear at τ 8.25. The NMR spectrum also showed two doublets at τ 2.05 (J 9 Hz) and τ 2.90 (J 9 Hz) due to two aromatic protons. The chemical shift of the low field doublet showed that it could only arise from a proton next to a xanthone carbonyl.



24



25

a. $R_1 = H, R_2 = R_3 = Me$
b. $R_2 = H, R_1 = R_3 = Me$
c. $R_3 = H, R_1 = R_2 = Me$

This must be either C-1 or C-8 position of the xanthone nucleus. The adjacent position C-2 (or C-7) must bear the other aromatic proton to account for the magnitude of the coupling. Also visible in the NMR spectrum were doublets at τ 3.46 (J 10 Hz) and τ 4.32 (J 10 Hz) and a sharp singlet at τ 8.41 (6H). These signals indicated the presence of a 2,2-dimethyl chromene system. The fact that the chromene double bond was conjugated with xanthone system was shown by the change of uv pattern of this pigment on complete hydrogenation. The presence of 2,2-dimethyl chromene system was also shown by an intense M-15 peak at *m/e* 407 (base peak) in the mass spectrum of kayeaxanthone. The peak was most probably due to the stable benzopyrylium ion formed from the 2,2-dimethyl chromene system.

The NMR spectrum of the methyl ether of kayeaxanthone showed the presence of three methyl groups while that of kayeaxanthone showed two methyl groups. This indicated the presence of one hydroxy group and two methoxy groups in kayeaxanthone.

Having identified the various substituents it was now left to orientate them about the xanthone nucleus. The two aromatic protons could occupy as mentioned above either of the equivalent positions 8 and 7 or 1 and 2 of the xanthone nucleus.

2-Methoxy-1,3,5-trihydroxyxanthone (21) was the only xanthone isolated from the timber extract. Biosynthetic analogy with this xanthone suggested the same oxygenation pattern for the bark chromenoxanthone**. Further the u.v. pattern of the hydrogenated chromenoxanthone is very similar to that of some of the known 1, 2, 3, 5-tetraoxygenated xanthone systems. (*Table* 5).

Table 5. Comparison of u.v. absorption maxima λ_{max} (ethanol) (log ε)

Kayeaxanthone hydrogenated	217 (3.89)	244 (3.92)		262^{sh} (3.82)	301 (3.83)	353 (3.55)
2-Methoxy-1, 3, 5-trihydroxyxanthone	222 (3.91)	244 (3.93)		262^{sh} (3.87)	314 (3.57)	365 (3.56)
1-Hydroxy-2,3,5-trimethoxyxanthone	220	242	253	258^{sh}	302	370

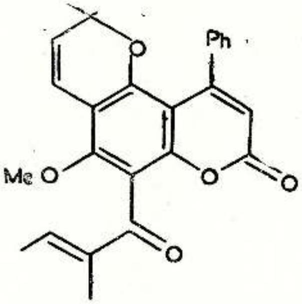
In keeping with the above arguments six possible structures (24a, b, c, and 25a, b, c) can be written for kayeaxanthone. The NMR spectrum of this xanthone did not show a low field signal of a chelated hydroxy group. Also the uv spectrum remained unchanged on addition of $AlCl_3$. Further methylation of the pigment with diazomethane as well as dimethyl sulphate gave the same methyl ether. All these observations indicated that position C-1 must bear a methoxy group rather than a hydroxy group. This rules out structures (24a) and (25a). The structure (25 c) is very unlikely as the uv spectrum of the original pigment remained unchanged on addition of sodium acetate. All attempts to cyclise the chromenoxanthone failed. This showed that 1,1-dimethyl-prop-2-enyl side chain cannot occupy a position ortho to a hydroxy group, since otherwise it would cyclise easily under mild acid conditions to give a dihydrofuran ring. This experiment rules out structures (24c) and (25c) leaving only (24b) and (25b) as the possible structures of this kayeaxanthone.

Further structural investigation of this xanthone is being delayed for want of plant material and due to the extreme difficulty in isolating the same. One method of distinguishing between the above two structures would be the use of nuclear overhauser effect technique.

** This argument may not be valid and is being further tested at the moment.

Coumarins

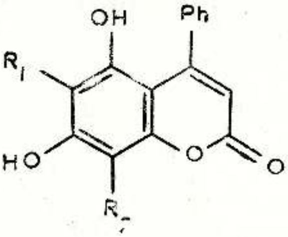
More than 50 coumarins have been isolated and characterised from various species of Guttiferae. The coumarins have been isolated mostly from the seeds and bark extracts. All the coumarins found in Guttiferae have a 5,7-oxygenated pattern and often have alkyl, acyl, hydroxyl group as substituents. There are also a few cases of coumarins with a methoxy group as a substituent, for example calophyllolide[28] (26)



26

Crombie[9] and co-workers have broadly classified these coumarins into a 6-acyl class and an 8-acyl class, largely based on the differences in base shifts in the ultra violet spectra of these compounds. The coumarins which belong to the 6-acyl class have an acyl substituent in position 6 as for example, mesuol (27e) and the coumarins which belong to the 8-acyl class have an acyl substituent in position 8 as for example mammea A/BB (27d).

Members of these two classes also differ in their colour reaction with neutral ferric chloride. These coumarins may also be divided into another two groups namely a 4-phenyl group : (27)



27

	R_1	R_2
(*a*) Mammea A/AA (mammeisin)	$CO.CH_2.CH.(CH_3)_2$	$CH_2.CH:C(CH_3)_2$
(*b*) Mammea A/AB	$CO.CH(CH_3).CH_2.CH_3$	$CH_2.CH:C(CH_3)_2$
(*c*) Mammea A/BA	$CH_2.CH:C(CH_3)_2$	$CO.CH_2.CH(CH_3)_2$
(*d*) Mammea A/BB	$CH_2.CH:C(CH_3)_2$	$CO.CH.(CH_3)CH_2.CH_3$
(*e*) Mesuol	$CO.CH.(CH_3)_2$	$CH_2.CH:C(CH_3)_2$

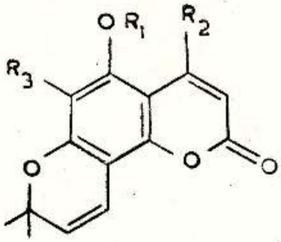
and a 4-alkyl group: (28)

28

$R_1 = CH_2CH_2CH_3$	R_2	R_3
(*a*) Mammea B/BC	$CH_2.CH:C(CH_3)_2$	$CO.CH_2.CH_2.CH_3$
(*b*) Mammein B/BA	$CH_2CH:C(CH_3)_2$	$CO:CH_2.CH(CH_3)_2$
(*c*) Mammea B/BB	$CH_2CH:C(CH_3)_2$	$CO.CH(CH_3)CH_2CH_3$
(*d*) Mammea B/AB	$CO.CH(CH_3)CH_2CH_3$	$CH_2CH:C(CH_3)_2$
(*e*) Mammea B/AA	$CO.CH._2CH(CH_3)_2$	$CH_2.CH:C(CH_3)_2$
(*f*) Surangin A	$CH_2.CH:C(CH_3)CH_2.CH_2.CH:C(CH_3)_2$	$CO.CH(CH_3)CH_2CH_3$
$R_1 = CH(CH_3).CH_2.CH_3$		
(*g*) Ferruol A	$CH_2CH:C(CH_3)_2$	$CO.CH(CH_3)CH_2.CH_3$
$R_1 = CH_2CH_2CH_2CH_2CH_3$		
(*h*) Mammea C/BB	$CH_2CH:C(CH_3)_2$	$CO.CH(CH_3)CH_2.CH_3$
$R_1 = CH(O.CO.CH_3)CH_2CH_3$		
(*i*) Surangin B	$CH_2.CH:C(CH_3)CH_2.CH_2CH:C(CH_3)$	$CO.CH(CH_3)CH_2CH_3$
(*j*)	$CH_2.CH:C(CH_3)_2$	$CO.CH_2.CH(CH_3)_2$
(*k*)	$CH_2.CH:C(CH_3)_2$	$CO.CH(CH_3)CH_2CH_3$

In fewer cases like surangin B(28i) acylated substituents are present in position 4 of the coumarin nucleus.

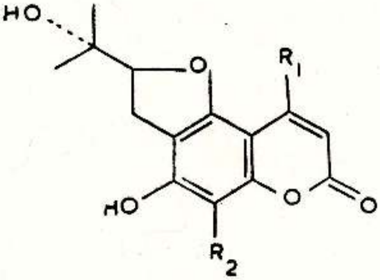
The isopentenyl substituents which are very common in the Guttiferae coumarins may occupy position 6 as in mammeisin (27a) or position 8 as in mammea B/BA (28b) depending on the position of the acyl group. Often these isopentenyl side chains undergo cyclisation with the orthohydroxy group to give a chromene ring as in the case of mesuagin (29e)



29

	R_1	R_2	R_3
(*a*) * Mab 5	H	Ph	$CO.CH(CH_3)CH_2.CH_3$
(*b*) Mab 6	H	$CH_2CH_2CH_3$	$CO.CH(CH_3)CH_2.CH_3$
(*c*) Apetalolide	CH_3	Ph	$CO.C(CH_3){:}CH.CH_3$
(*d*) * Mammeigin	H	Ph	$CO.CH_2.CH(CH_3)_2$
(*e*) * Mesuagin	H	Ph	$CO.CH(CH_3)_2$

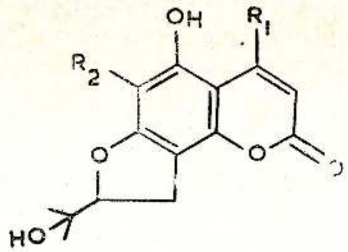
Such cyclisation can also lead to a hydroxyisopropyldihydrofuran system as in the case of coumarin (30)



30

R_1	R_2
$CH_2.CH_2.CH_3$	$CO.CH_2.CH(CH_3)_2$
$CH_2.CH_2.CH_3$	$CO.CH(CH_3).CH_2.CH_3$
$CH_2.CH_2.CH_3$	$CO.CH_2.CH_2.CH_3$

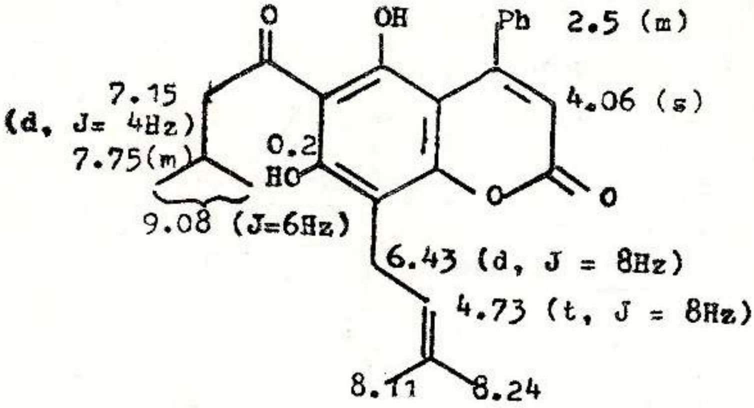
or to a 2,2-dimethyl-3-hydroxydihydropyran system as in the case of coumarin (31)



31

	R_1	R_2
(*a*) *	Ph	$CO.CH_2.CH(CH_3)_2$
(*b*) * Mab 3	Ph	$CO.CH(CH_3).CH_2.CH_3$
(*c*)	$CH_2.CH_2.CH_3$	$CO.CH_2.CH(CH_3)_2$
(*d*) Mab 4	$CH_2.CH_2.CH_3$	$CO.CH(CH_3).CH_2.CH_3$
(*e*) *	Ph	$CO.CH(CH_3)_2$

From the bark extracts of *Mesua thwaitesii* Planch & Triana a coumarin was isolated. Its uv spectrum showed a characteristic base shift for a 6 acyl coumarin and the NMR data fixed the structure as 4′, 5′-dihydro-5-hydroxy-5′-(1-hydroxy-1-methylethyl)-6-(3-methylbutyryl)-4-phenylfuro-[2′, 3′: 7,8] coumarin (31a)



4-phenyl-5,7-dihydroxy-6-isovaleryl-8-isopentenyl coumarin

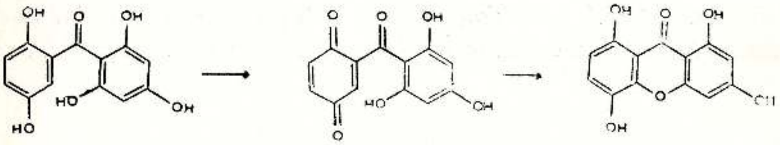
32

From the light petroleum extract of the seeds of *Mesua thwaitesii* Planch & Triana mammea A/AA (mammeisin) 4-phenyl-5, 7-dihydroxy-6-isovaleryl-8-isopentenyl coumarin (27a) and the coumarin isolated from the bark (31a) were obtained in a pure state. The NMR data for mammeisin is given in (32). By a G.C. - mass spectrometric examination by Dr. D. E. Games[9] at the University of Wales, Cardiff, the presence of five other coumarins 29a, 29d, 29e, 31b and 31e were established. Coumarin (31e) is being reported for the first time.

Xanthone formation mechanisms

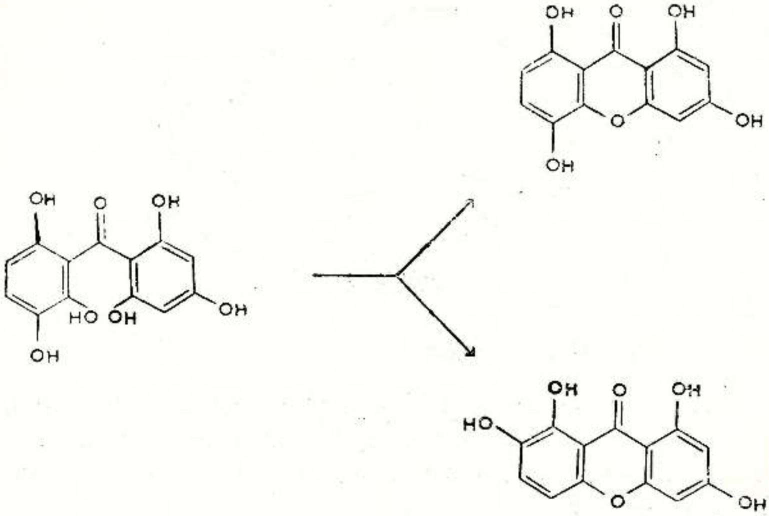
It has been established that xanthones in higher plants arise by condensation of acetate and shikimate derived moieties. It is believed that this condensation results in benzophenones which undergo intramolecular reaction to give the xanthones. Benzophenones are in fact known to co-occur with xanthones in some plants.

A number of mechanisms have been proposed for the intramolecular reaction of benzophenone leading to xanthone. One such mechanism involves a quinone intermediate[11] (*Scheme* 1)



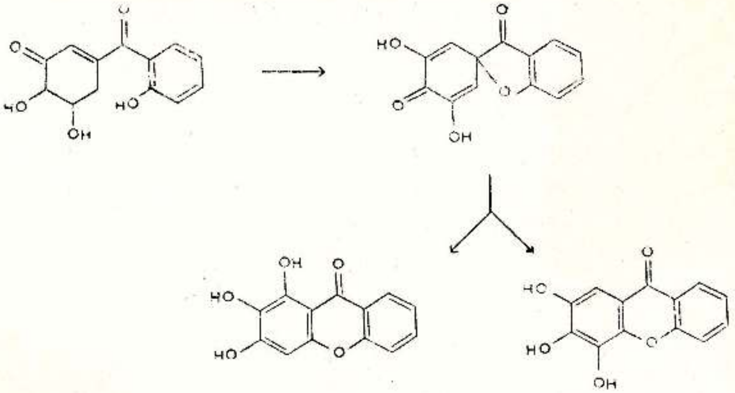
Scheme 1

Seshadri[26] and others postulated that xanthones could arise by dehydration between hydroxy groups of the acetate and shikimate derived moieties (*Scheme* 2).



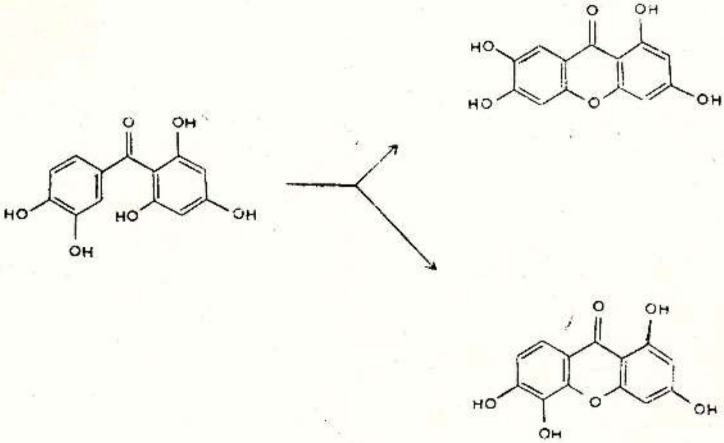
Scheme 2

A biogenetic pathway for xanthone formation from benzophenone involving a spirodienone intermediate (*Scheme* 3) has been suggested by Gottlieb[12]



Scheme 3

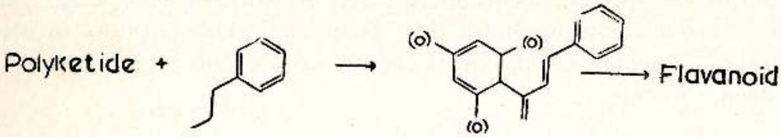
but Scheinmann[7] and co-workers favour a direct phenol oxidative coupling mechanism for the formation of xanthones from benzophenone (*Scheme* 4)



Scheme 4

Neoflavanoid formation mechanisms

Various biogenetic theories have been proposed regarding the formation of neoflavanoids in plants. One such theory[36] suggests that these neoflavanoids arise by a biogenetic pathway where 1,2-aryl shifts from a chalcone precursor takes place (*Scheme* 5)



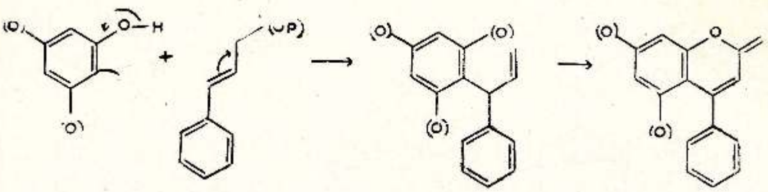
$\xrightarrow[\text{shifts}]{\text{one 1-2-aryl}}$ Isoflavanoid

$\xrightarrow[\text{shifts}]{\text{two 1, 2-aryl}}$ Neoflavanoid

Scheme 5

This biogenetic pathway is similar to the one established for flavanoids and isoflavanoids. Seshadri[30] suggested that neoflavanoids could arise by another mode of attachment of the C_9 unit with the phenolic unit.

Ollis[27] and co-workers suggested that cinnamyl pyrophosphate could be the C_9 compound and this unit could alkylate a phenolic unit or its polyketide equivalent to form dalbergiones which in turn oxidise to give 4-phenyl coumarins (*Scheme* 6)

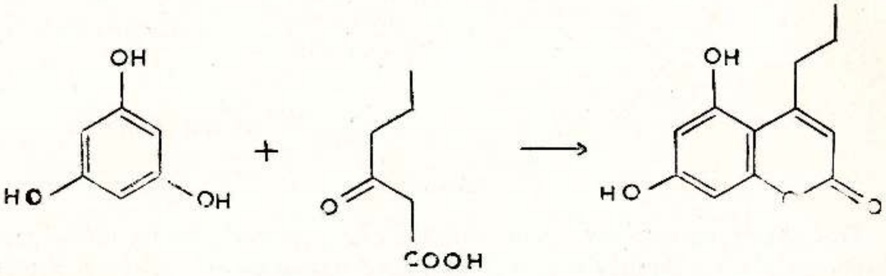


Scheme 6

Ollis, Gottlieb[13] and co-workers later suggested that isomerisation to 1-phenylallylpyrophosphate takes place before neoflavanoid formation.

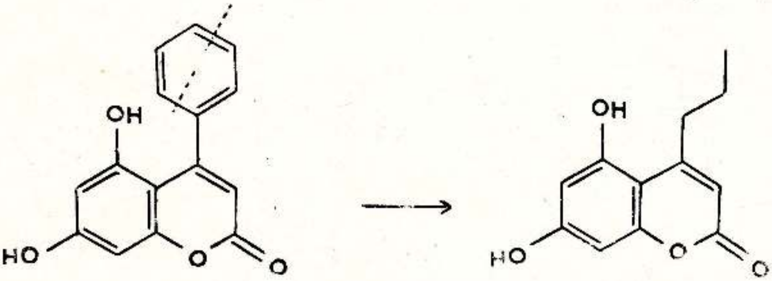
Kunesh and Polonsky[22] carried out feeding experiments on *Calophyllum inophyllum* L. seeds with specifically labelled phenyl alanine. They isolated calophyllolide (26) and ruled out the first theory where the biogenetic pathway is similar to that established for flavanoids and isoflavanoids. They also established that the C_9 unit in the formation of neoflavanoids is formed by shikimate-prephenic pathway. Later the same group performed some feeding experiments[23] using L-U- ^{14}C isoleucine, L-U- ^{14}C leucine and 1-^{14}C acetate and showed L - U - ^{14}C isoleucine is specifically incorporated into the tigloyl side chain of calophyllolide (26). They also noted that 1- ^{14}C acetate, apart from being incorporated into the phloroglucinol ring, was also incorporated into carbon atom C-3 and C-4 of the tigloyl grouping.

Apart from 4 phenyl coumarins, alkyl coumarins have been isolated from the Guttiferae species. Scheinmann[8] and co-workers have suggested two possible biogenetic pathways for their formation. One pathway involves an addition of a phenolic C_6 unit or its equivalent to a keto acid or its polyketide equivalent (*Scheme* 7)



Scheme 7

The second pathway is by biodegradation of the phenyl coumarin (*Scheme* 8) to give 4 alkyl coumarins.



Scheme 8

This pathway probably accounts for the fact that most alkyl substituents of the coumarins isolated from Guttiferae have not exceeded five carbon atoms and also these alkyl substituents may be branched as in ferruol A (28*g*). Feeding experiments using specifically labelled shikimate moieties may throw some light on the biogenesis of these four alkyl coumarins.

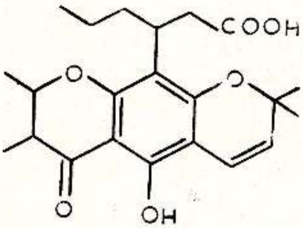
Cuneifolic acid

From the *Calophyllum cuneifolium* Thw. bark extracts, we have isolated a new acid which we have called cuneifolic acid. The uv spectrum resembles that of apetalic and blancoic acids (*Table* 6)

Table 6. Comparison of uv spectra λ_{max} (log ε) nm

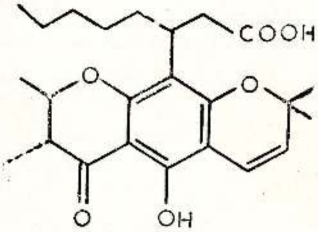
Cuneifolic acid	268 (4.49)	276 (4.53)	301 (4.01)	314 (4.03)	368 (3.77)
Apetalic acid[14](33)	268 (4.49)	276 (4.53)	301 (4.40)	315 (4.03)	368 (3.37)
Blancoic acid[35](34)	267 (4.60)	275 (4.62)	300 (4.05)	312 (4.07)	365 (3.33)

The NMR spectrum of cuneifolic acid gave valuable information on its structure especially when compared to those of apetalic (33), blancoic (34) and other related acids from species of *Calophyllum* L.



Apetalic acid
(2,3 methyl *cis*)

33



Blancoic acid
(2,3 methyl *trans*)

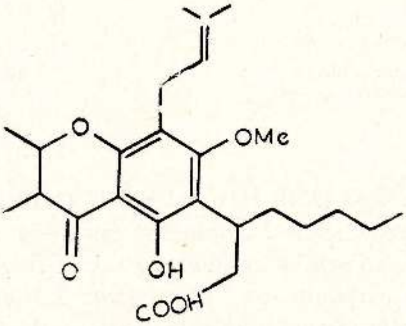
34

The doublets at τ 3.43 (J 10 Hz) and τ 4.43 (J 10 Hz) and the singlet at τ 8.58 are characteristic of a 2,2-dimethyl-chromene system. The presence of this system is also shown by an intense peak at *m/e* 373 (M-15), most probably due to M-15 pyrylium ion formed from 2,2-dimethylchromene. The presence of a propylpropionic acid side chain in this acid as in the case of apetalic acid (33) is shown by signals at τ 6.28 (1H,*m*), τ 7.25 (2H,*m*), τ 8.64 (4H broad) and τ 9.13(3H,*t*) in the NMR spectrum.

The presence of a 2,3-dimethylchromanone system is shown by the signals at τ 5.80 (1H,*m*), τ 7.32 (3H,*d*) and τ 8.82(3H, *d*). The NMR spectrum of this acid is somewhat similar to that of apetalic acid (33) suggesting close structural similarities between these two acids. But a significant difference is seen in the signals of the 2,3-dimethyl-chromanone system. In apetalic acid where the two methyl groups of the chromanone system have cis-relationship to each other, the multiplet of the C-2 proton of the chromanone system appears at τ 5.5 whereas in cuneifolic acid this multiplet appears at τ 5.80. Comparison of the chemical shifts of C-2 proton multiplet and C-2 and C-3 methyl signals of the chromanone system of this acid with the chemical shifts of *cis*- and *trans*- 2,3-dimethylchromanone of known compounds (*Table* 7) suggested that the two methyl groups in cuneifolic acid have a trans-relationship to each other.

Table 7. Chemical shifts as τ values

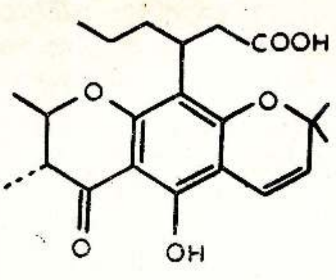
	C-2 proton	C-2 *and* C-3 methyls	
1. Papuanic acid [33](*trans*-) (35)	5.88	8.51	8.84
2. Isopapuanic acid[33] (*cis*-) (36)	5.44	8.63	8.82
3. Brasiliensic acid [34](*trans*-)	5.85	8.48	8.79
4. Isobrasiliensic acid [34](*cis*-)	5.50		
5. Apetalic acid (*cis*-) (33)	5.50	8.69	8.91
6. Blancoic acid (*trans*-) (34)	5.96	8.52	8.82
7. Cuneifolic acid (*trans*-)	5.80	8.50	8.82



(35) Papuanic Acid (2, 3 methyl *trans*)

(36) Isopapuanic Acid (2, 3 methyl *cis*)

Having identified the various substituents of the *cis*-2,3-dimethylchromanone system it is now left to orientate them about the chromanone ring. Biosynthetic analogy with coumarins suggested a phloroglucinol oxygenation pattern for this acid. Further evidence of this oxygenation pattern comes from close similarity of the uv pattern of this acid to those of blancoic acid and apetalic acid. Placing the oxygen atoms in keeping with the above arguments two structures (37) and (38) are possible for cuneifolic acid.



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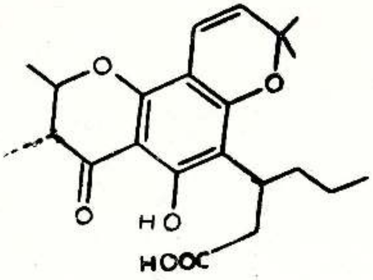

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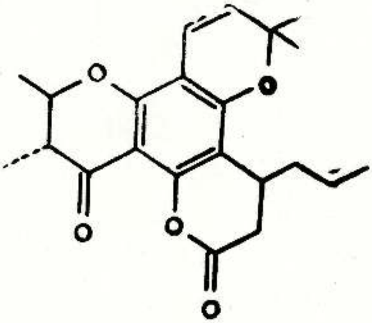


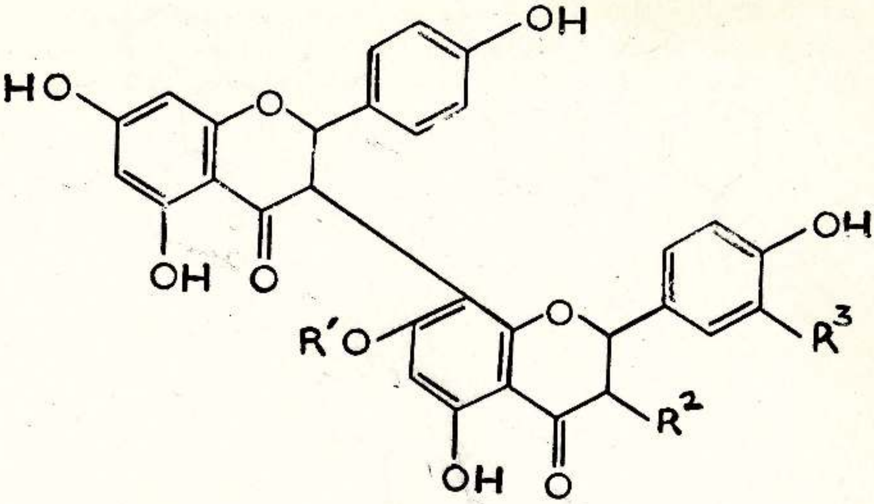
39

The two structures could be easily distinguished. Cuneifolic acid would readily lactonise to give the product (39) if it had the structure (38). NMR spectrum of the acetate of cuneifolic acid would also give some information on the orientation of the substituent about the chromane nucleus. Treatment of the acid with acetic anhydride and pyridine gave a gum. The gum which was homogeneous on TLC gave a strong peak at 1785 cm^{-1} in its ir spectrum. This absorption is characteristic of the phenyl esters. The shift of ir absorption from 1700 cm^{-1} in the acid to 1785 cm^{-1} in the gum strongly suggested that lactonization has taken place as in the case of papuanic acid (35). Since lactonization had probably taken place, cuneifolic acid must probably have the structure (38). Further confirmation of the structure of cuneifolic acid must await the NMR spectra of the gum.

Biflavanoids

Three types of biflavanoids have been described from the bark and timber of Guttiferae species.

1. Biflavanoid type where both monomers are flavanones like naringenin, aromadendrin, taxifolin etc. *eg*. II-3, I-4′, II-4′ I-5, II-5, I-7, II-7 heptahydroxy-[I-3, II-8] biflavanone (40). These biflavanoids are referred to as GB biflavanoids[17].

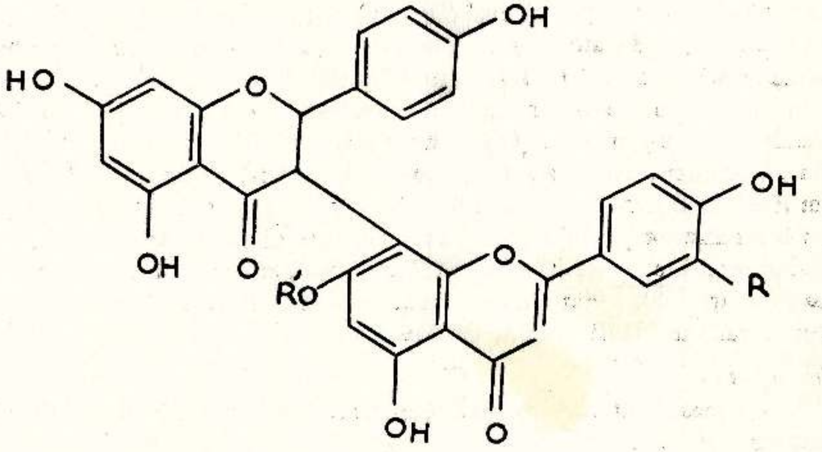


40

(*a*) $R^1 = H$, $R^2 = OH$, $R^3 = OH$, GB-2
(*b*) $R^1 = H$, $R^2 = OH$, $R^3 = H$, GB-1 — II-3, I-4′, II-4′, I-5, II-5, I-7, II-7 heptahydroxy-[I-3, II-8] biflavanone

(*c*) $R^1 = R^2 = R^3 = H$, GB-1a
(*d*) $R^1 = R^2 = H$, $R^3 = OH$, GB-2a
(*e*) R^1 = glucose moiety $R^2 = H$, $R^3 = OH$

2. Flavanone : flavone type where one unit is naringenin (42) and the second unit is flavone, *e.g.* volkensiflavone[17] (41b).



41

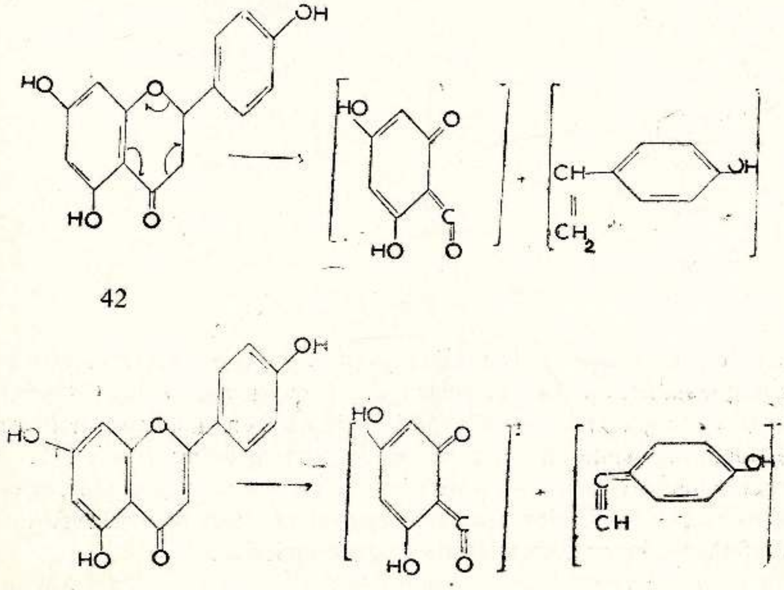
(*a*) $R = OH$, $R^1 = H$, morelloflavone
(*b*) $R = R^1 = H$ volkensiflavone: I-4′, II-4′, I-5, II-5, I-7, II-7 hexahydroxyflavanone [I-3, II-8] flavone
(*c*) $R = H$, R^1 = glucose moiety – spicataside
(*d*) $R = OH$, R^1 = glucose moiety – fugugiside

3. Biflavanoid glycosides eg. fugugiside[30] (41d)

All the biflavanoids isolated from the timber, bark and root extracts of Guttiferae species have a [I-3, II-8] interflavanoid linkage.

The separation of the biflavanoids in the two species studied *Garcinia terpnophylla* Thw. & *Garcinia echinocarpa* Thw. presented considerable difficulties as no separation could be effected on silica gel or cellulose columns. Therefore partial separations were effected by cold aqueous sodium carbonate and borax extraction. The borax extracts were taken up in ethyl acetate and then partitioned in a counter current distribution unit. The fractions from these were then separated on a polyamide column to give each of the biflavanoids F_1 , F_2 and F_3 from *G. terpnophylla* Thw. in a pure state.

All three pigments from *G. terpnophylla* Thw. were pale yellow in colour and gave a deep red colouration with magnesium turnings and conc. hydrochloric acid. They all had similar uv patterns and their maxima showed, on addition of sodium acetate the bathochromic shifts characteristic of 5,7-dihydroxyflavanone system. Their molecular weights as determined by mass spectrometry indicated that they are flavanoid dimers. The study of the physical data particularly the mass spectra of pigments F_1 , F_2 and F_3 led to the identification of these flavanoid dimers. Simple flavones and flavanones are known to undergo a retro Diels-Alder fragmentation in their mass spectra as for example naringenin (42) and apigenin (43) (*Scheme* 9).



42

43

Scheme 9

A similar process was observed in the case of volatile biflavanoids where two successive retro Diels-Alder fragmentation takes place *eg*. II-3, I-4′, II-4′, I-5, II-5, I-7, II-7 heptamethoxy-[I-3, II-8] biflavanone (*Chart* 1)

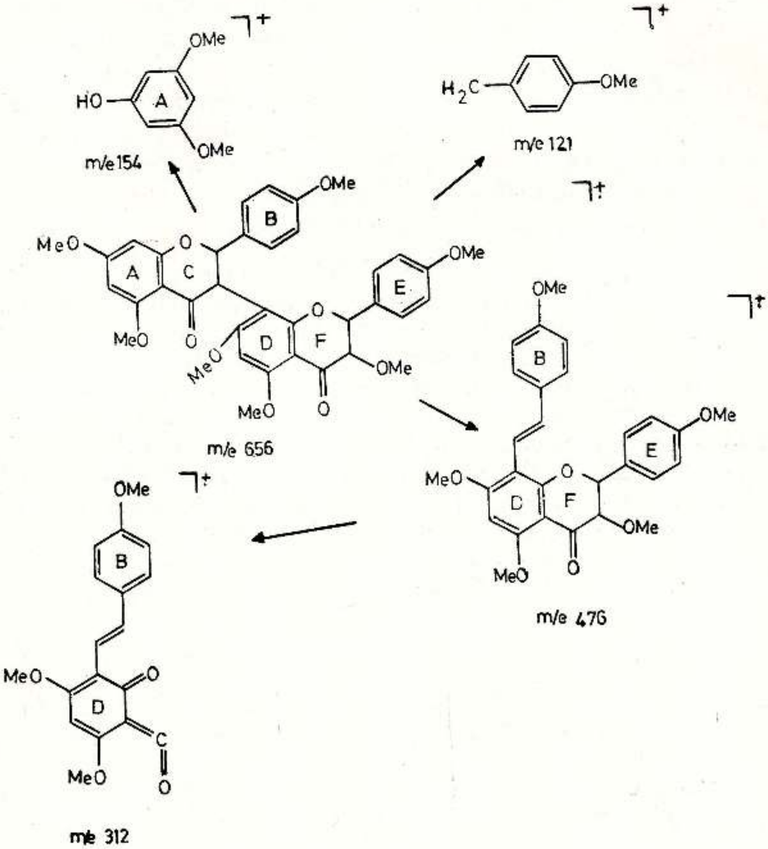


Chart 1

The base peak at *m/e* 312 is a direct result of the two successive retro Diels-Alder fragmentation of the molecular ion. The cleavage of ring C takes place first to give a fragment of *m/e* 476 (M-180) which then undergoes another retro Diels-Alder fragmentation to give the ion corresponding to *m/e* 312. Also observed in the spectra are p-methoxy benzyl ion of *m/e* 121 and phloroglucinol ion of *m/e* 154. The phloroglucinol fragment of *m/e* 154 is believed to be a result of thermal breakdown of the parent molecule.

The appearance of phloroglucinol fragment and p-methoxy benzyl fragment in the mass spectrum of the above heptamethyl ether confirms that (*a*) at least one phloroglucinol ring is not included in the linkage of the two monomer units and that

(*b*) both heterocyclic rings have a methoxy phenyl substituent and these methoxy phenyl substituents are not involved in the linkage of the two flavanoid units. It has been observed that volatile biflavanoids like GB 1a (F_1) (*Chart* 2)

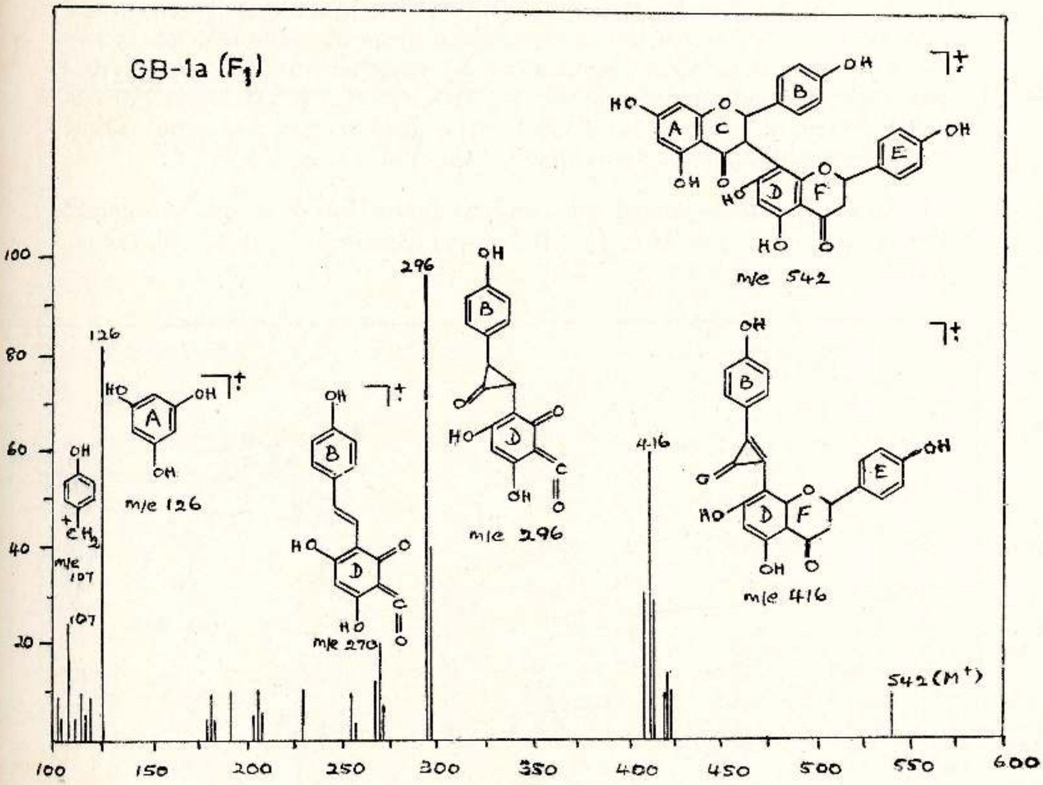


Chart 2

lose a molecule of phloroglucinol very readily from the parent ion and give rise to a base peak at *m*/*e* 126. The loss of phloroglucinol from the parent ion is often followed by a single retro Diels-Alder fragmentation.

The pigment F_1 showed the u.v. maxima at 228 (log ε 4.52), 290(4.49) and 335 (3.92) nm. In the mass spectrum the molecular ion appeared at *m*/*e* 542 (*Chart* 2). The peaks at *m*/*e* 126 and *m*/*e* 107 most probably due to phloroglucinol ion and p-hydroxybenzyl ions respectively, suggested that at least one phloroglucinol ring is not involved in the interflavanoid linkage and that one of the hetrocyclic rings had a hydroxy phenyl substituent and this hydroxyphenyl substituent is not involved in the interflavanoid linkage. The u.v. pattern and the presence of these peaks in the mass spectrum indicated that F_1 was a GB flavanoid. The loss of elements of phloroglucinol from the parent ion gave an ion of *m/e* 416 which then underwent retro Diels-Alder fragmentation to give an ion of *m/e* 296. The absence of a peak due to a dihydroxybenzyl fragment at *m/e* 123 indicated that both heterocyclic rings probably had only hydroxyphenyl substituents. Also visible in the mass spectrum is a weak peak at *m/e* 270 which could have arisen from the molecular ion by two successive retro Diels-Alder fragmentations. Together with the other physical data, these data indicated that F_1 was I-4′, II-4′, I-5, II-5, I-7, II-7 hexahydroxy-[I-3, II-8] biflavanone (GB 1a)[26] (40c). This identification was confirmed by comparison with an authentic sample *i.r.* and TLC

From the spectroscopic and mass spectral data (*Chart* 3) F_2 was recognized as II-3, I-4′, II-4′ I-5, II-5, I-7, II-7 heptahydroxy-[I-3, II-8] biflavanone (GB 1)[17] (40 b).

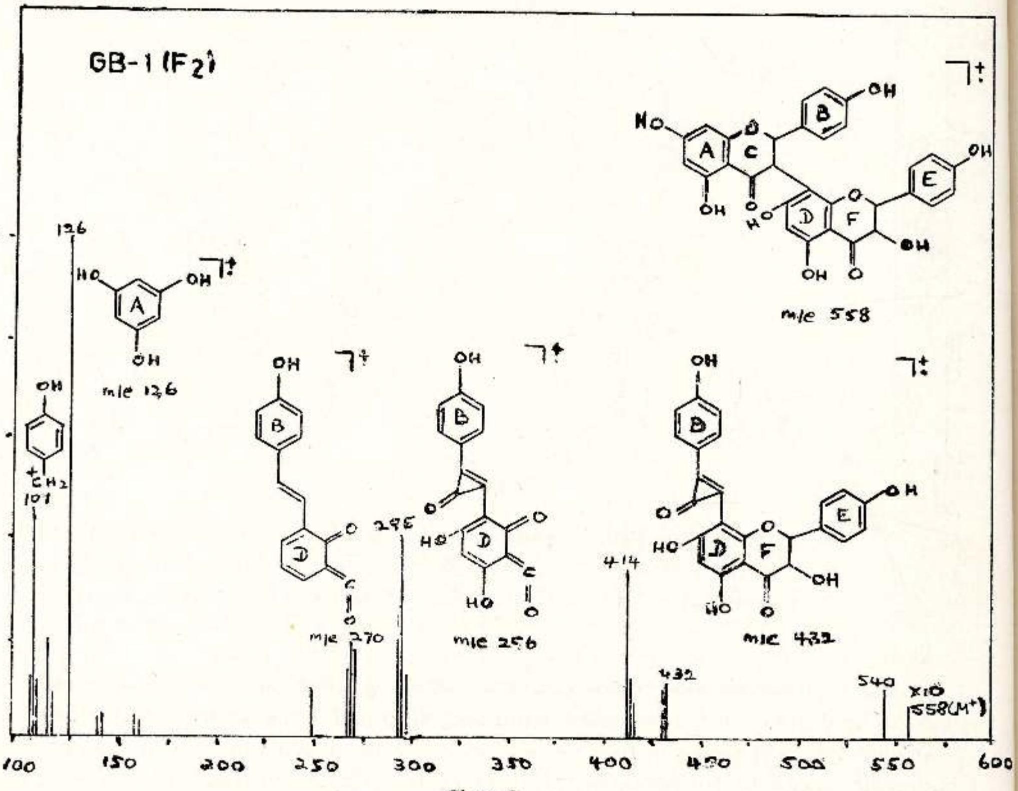


Chart 3

This identification was confirmed by comparison with an authentic sample *ir* and TLC)

The third biflavanoid F_3, in a similar manner (*Chart* 4) was identified as II-3, II-3′ I-4′ II-4′ I-5, II-5, I-7, II-7 octahydroxy [I-3, II-8] biflavanone (GB 2)[26] (40a) and confirmed by comparison with an authentic sample ir and TLC)

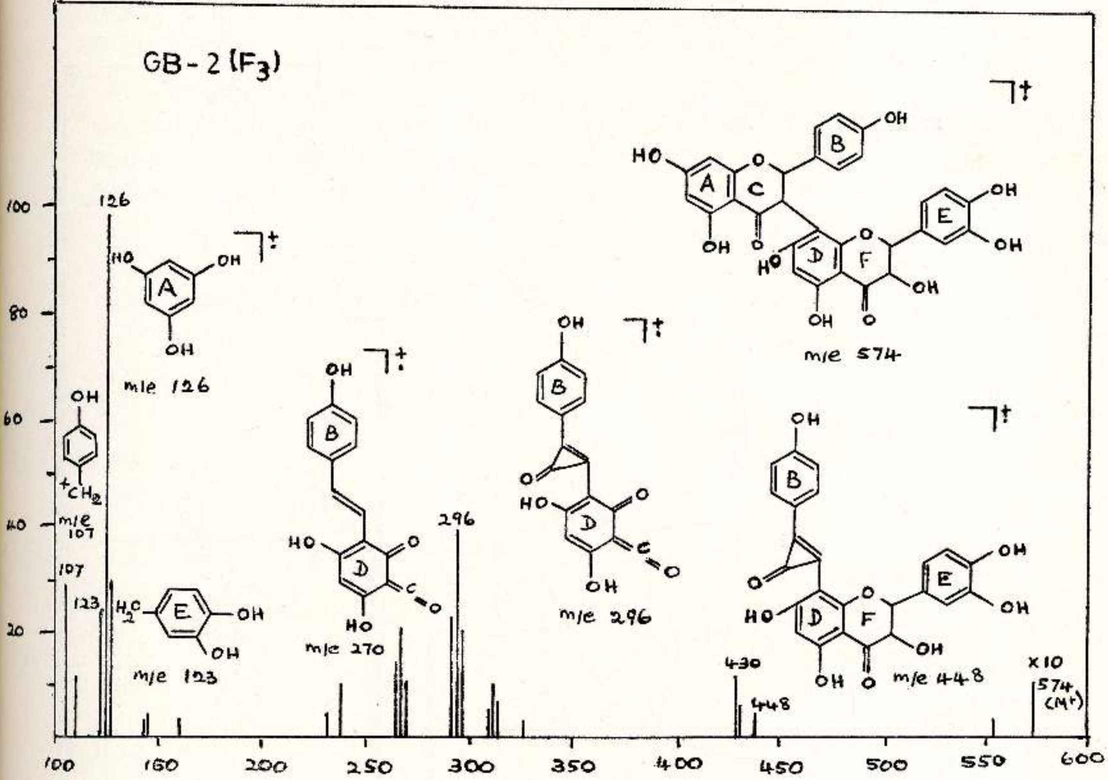


Chart 4

Both pigments F_4 and F_6 from *G. echinocarpa* Thw. gave green colouration with neutral ferric chloride solution and deep red colouration with magnesium turnings and conc. hydrochloric acid. Besides the pigments showed uv patterns similar to that expected for a combination of naringenin (42) and apigenin (43) (*Table* 8)

Table 8. **uv absorption maxima λ_{max} ethanol**

Flavanoids	uv maxima in ethanol	λmax (log ε) nm		
Pigment F_4	225(4.60)	275(4.38)	289(4.44)	330(4.20)
Pigment F_6	225(4.57)	275(4.38)	288(4.33)	340(4.25)
Apigenin		268(4.23)		335(4.40)
Naringenin			288(4.25)	330(3.68)

The biflavanoid F_6, molecular weight 556, showed characteristic peaks at *m/e* 126, 107 and at *m/e* 121 in its mass spectrum (*Chart* 5)

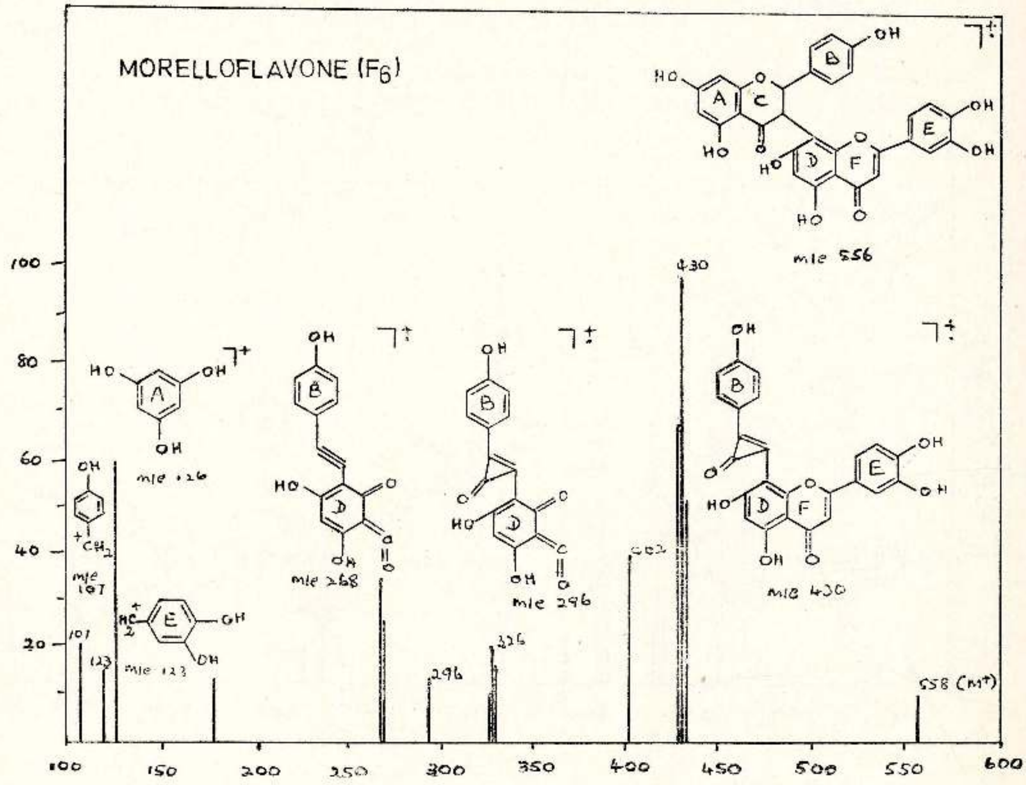


Chart 5

most probably due to phloroglucinol, parahydroxybenzyl and dihydroxybenzyl fragments respectively. These peaks and the uv data indicated that F_6 had the same carbon skeleton as the other Garcinia biflavanoids. The uv pattern (*Table* 8) suggested that one of the monomers was flavanone. The peak at *m/e* 107 and *m/e* 121 indicated that while one of the heterocyclic rings had a *p*-hydroxybenzyl substituent, the other had a dihydroxyphenyl substituent. In the mass spectrum of this pigment the most intense peak was at *m/e* 430 which probably arises from the loss of elements of phloroglucinol from the parent ion. The fragment either loses elements of carbon monoxide to give a fragment at *m/e* 402 and then undergoes retro Diels-Alder reaction to give a fragment at *m/e* 265 or first undergoes a retro Diels-Alder fragmentation to give a fragment at *m/e* 296 which subsequently loses carbon monoxide. From these mass spectral fragmentation pattern and other physical data, the pigment was identified as II-3′, I-4′, II-4′, I-5, II-5, I-7, II-7-heptahydroxyflavanone [-I-3, II-8] flavone (morelloflavone)[19] (41a). This identification was confirmed by comparison with an authentic sample (mixed mp., ir and T.L.C.).

The biflavanoid F_4 from its mass spectral fragmentation (*Chart* 6) and other physical data was identified as I-4′, II-4′, I-5, II-5, I-7, II-7 hexahydroxyflavanone [I-3, II-8] flavone (volkensiflavone)[15] (41b) and confirmed by comparison with an authentic sample ir and TLC).

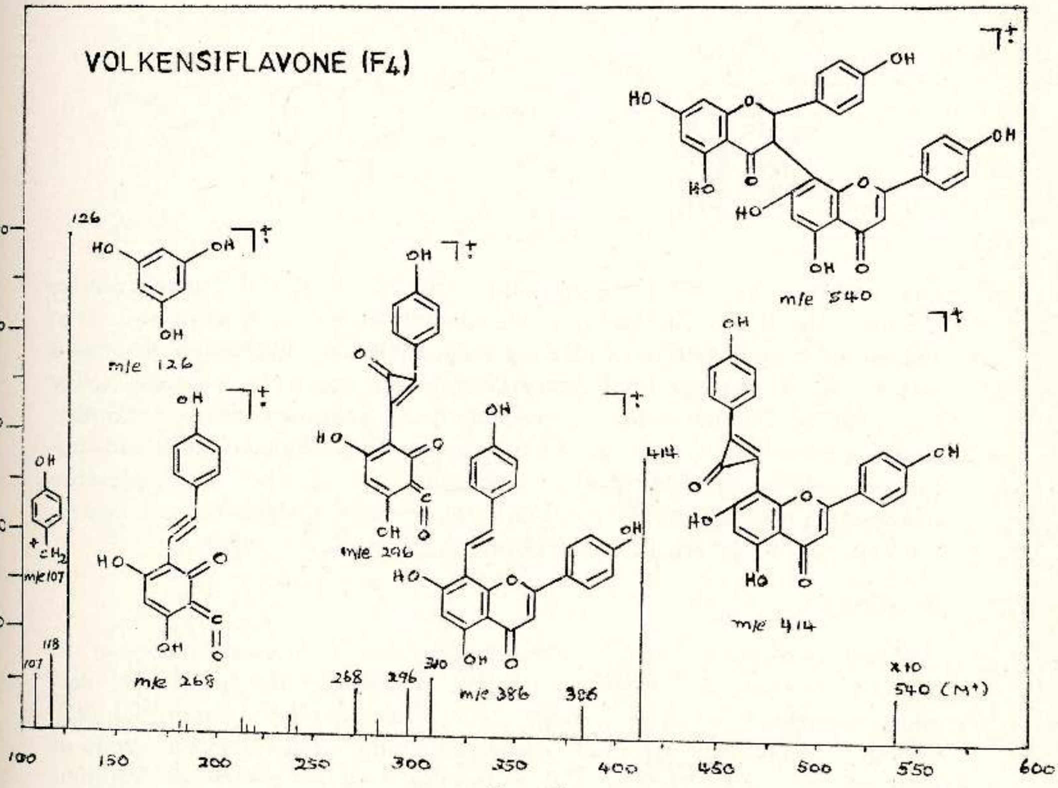
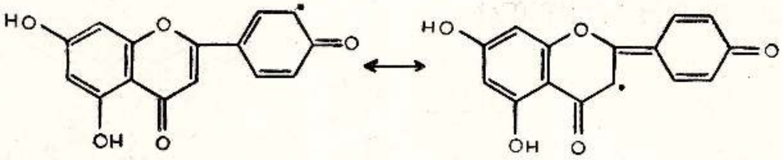


Chart 6

It has been suggested that biogenesis of these biflavanoids involves either a radical pairing or radical substitution of two complete embryonic flavanoid units[18]. The biogenetic pathway of flavanoids involves a combination of two fundamental pathways to aromatic compounds, namely shikimate and acetate pathways. The former pathway leads to the formation of ring B and C of the flavanoid. Studies using ^{14}C – labelled shikimic acid had shown that shikimic acid is incorporated in the ring B of quercetin. The ring A arises by the acetate pathway. The presence of hydroxy groups in positions 5 and 7 of the ring A in several flavanoids fits in with the phloroglucinol pattern. Also feeding experiments involving $C^{14}H_3COOH$ and $CH_3C^{14}OOH$ have shown that ring A of quercetin arises from acetic acid[5].

These biflavanoids are thought to arise when a quinone methide anion combined with an aryl system and the product enolises. Molyneux [25] *et. al.* have shown that in the case of apigenin the radical which is initially formed at C-4 oxygen atom on aerial alkaline oxidation is delocalised primarily to the C-3 and C-3′, positions (*Scheme* 10)

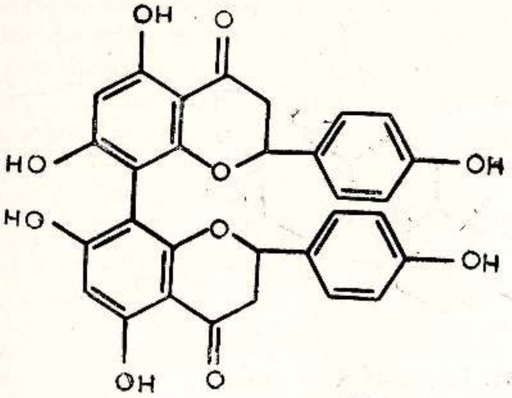


Scheme 10

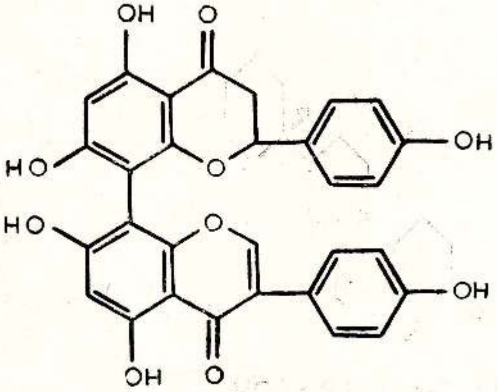
and that dimerisation leads to products with [3-3] and [3-3′] interflavanone linkage. Hence the formation of biflavanoids with [I-3, II-8] or [I-3, II-6] linkage by radical pairing in alkaline medium is very likely. Scheinmann *et al.*[18] however suggest that since Guttiferae timbers are invariably acidic there may be different modes of coupling under acidic conditions. Another probable mode of formation of these biflavanoids is by electrophilic substitution of a quinone methide radical on a phloroglucinol nucleus[5]. The presence of two other biflavanoids (44) and (45) in the stamens of *Mesua ferrea* L flowers was reported at the 8th IUPAC meeting[32] in New Delhi (1972).

Triterpenoids

Many triterpenes and steroids have been isolated from several species of the family Guttiferae. The triterpenes include members of the friedelane, oleanane, taraxerane, adianane groups etc. Friedelin (46), canophyllal (47), Canophyllol (48) etc. represent friedelane group. The oleanane group is represented by β-amyrin (49) and erythrodiol-3-acetate (50) while betulinic acid (51) and lupeol (52) represents the lupane group. Taraxerol (53) and tara-

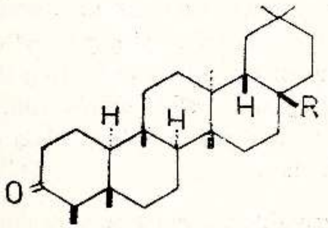


44 Mesua Ferrone A

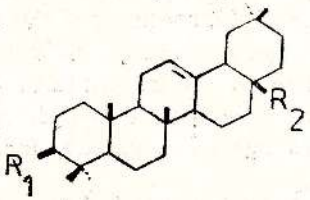


45 Mesua Ferrone B

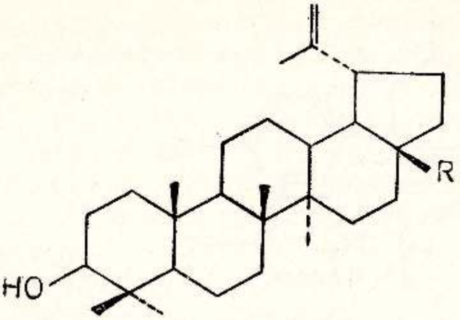
xerone (54) present in many *Calophyllum* L. species are members of the taraxerane group. An uncharacterised triterpene isolated from many *Calophyllum* L. species which is identified in the present work as simiaren-3 β-ol (55) belongs to the adianane group.



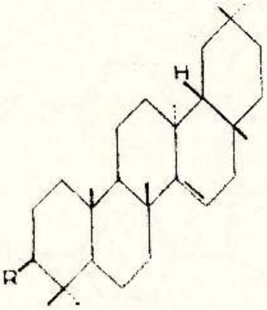
46 Friedelin $R=CH_3$
47 Canophyllal R=CHO
48 Canophyllol $R=CH_2OH$

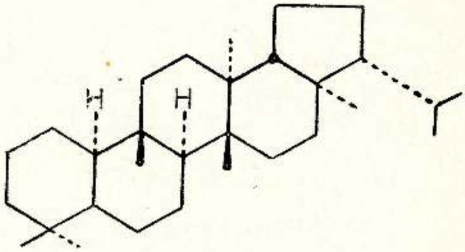


49 β Amyrin $R_1=OH$, $R_2=CH_3$
50 Erythrodiol-3-acetate
$R_1=OAc$, $R_2=CH_2OH$



51 Betulinic acid R=COOH
52 Lupeol R=CH_3





53 Taraxerol R = OH
54 Taraxerone R = O

55 β—simiarenol

In the plants investigated by us we have isolated friedelin (46), taraxerol (53), taraxerone (54) and β-simiarenol (55). The molecular formula of simiarenol was found to be $C_{30}H_{50}O$. The i.r. spectrum showed the presence of hydroxy group. This was also shown by its ready conversion into an acetate. The NMR spectrum of this triterpene showed the presence of eight methyl groups and a single olefinic proton (τ 4.40 complex multiplet) indicating the presence of a trisubstituted double bond. The signal at τ6.56 arises from a proton geminal to a hydroxy group. The small half width ($W_{\frac{1}{2}}$ 10 Hz) of this signal is suggestive for an equatorial proton.

The mass spectrum of this compound gave valuable information regarding its structure. Djerassi[6] and others have shown that the molecular ions of unsaturated triterpenes undergo a retro Diels-Alder fragmentation and furnish an intense peak. The base peak at *m/e* 274 of this triterpene could possibly arise from such a fragmentation. The mass spectral fragmentation of this triterpene showed a close resemblance to that of glutinone[21] (57). This showed that the triterpene has some structural similarities to that of glutinone (57). The intense peak at *m/e* 274 suggested that the double bond is in position 5 and the peaks at m/e 152 and *m/e* 134 (*Chart* 7) are in accordance with the glutinone type of skeleton.

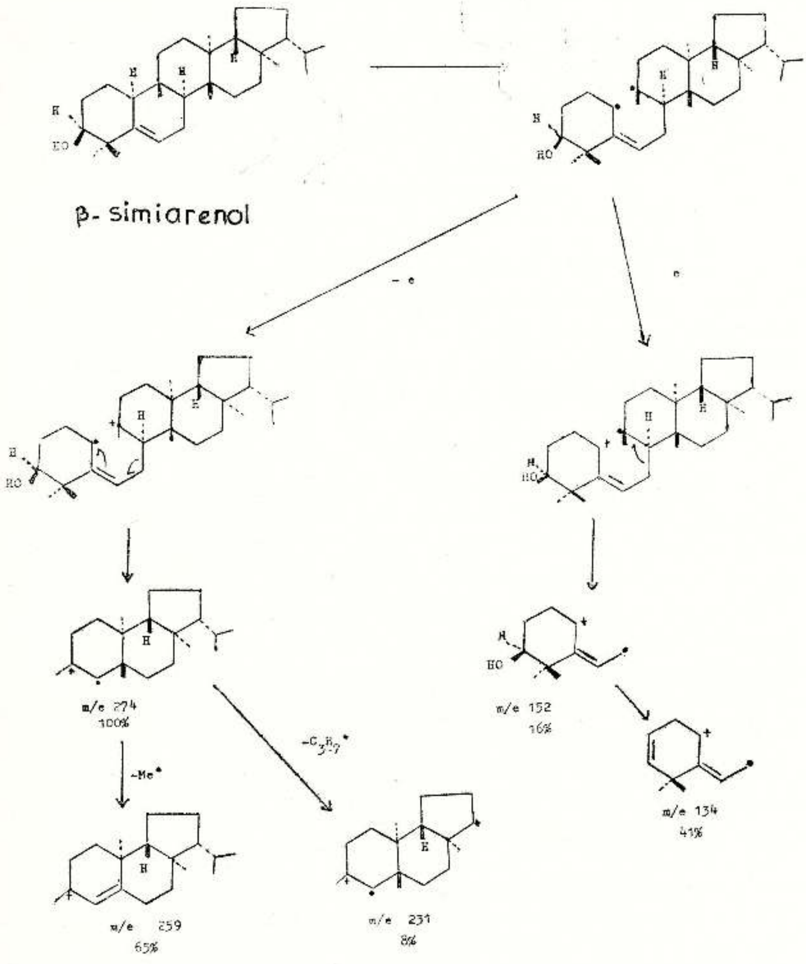
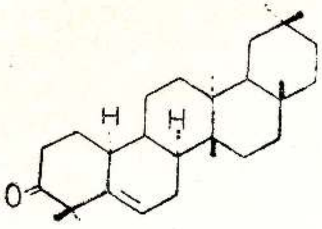
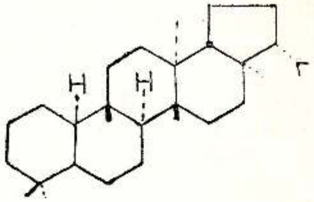


Chart 7

But a significant difference between the glutinone mass spectrum of this triterpene is the presence of M-43 fragment and a fragment at *m/e* 231 (*m/e* 274-43). These fragments suggested the presence of an isopropyl group. The presence of an isopropyl group in turn suggested that this triterpene belonged to the adianane (58) series rather than the glutinone series[2]. From these data and from the m.p. and rotation, this triterpene was identified as simiaren-3β-ol[2] (55). This identification was confirmed by comparison with an authentic sample (mixed mp., ir and T.L.C.).



57 Glutinone



58 Adianane

The acetate of this triterpene was shown to be identical to the acetate of simiaren-3β-ol by comparison with an authentic sample (mixed mp., ir and T.L.C.). The triterpene was oxidised with chromium trioxide-pyridine and the oxidant was shown to be identical to simiarenone (56) by comparison with an authentic sample (mixed mp., ir and T.L.C.).

This is only a general survey of the solid constituents present in some members of the family Guttiferae. Several chemotaxonomic correlations have also come out, but this would take far too long a time for elaboration and I shall omit it for the present. These studies have thrown out fresh problems that should be undertaken here as it would be most appropriate. I may mention just a few of them as a stimulus for further research in this field. The first species studied *Calophyllum calaba* L. is found in the Southern Province, Batticaloa area and even in Passara at an elevation of 4000 ft. The form of these trees is different and chemical investigation should show up important differences. Another is *Garcinia echinocarpa* Thw. (madol) which also seems to be different according to the location. In the current literature too there is a controversy at the moment on the constituents in *C. inophyllum* L. We too have started a study on it but the results are incomplete.*

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* The presence of jacareubin has been established

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Groundwater Recharge at Yala

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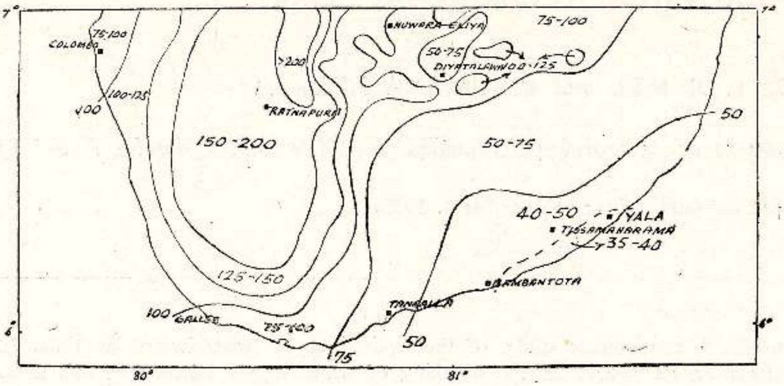
Abstract A preliminary study of the availibility of groundwater at Yala. The results could be utilized in the planning of water supply schemes in this area.

Groundwater recharge is often computed as a constant percentage of the annual rainfall. Due to the wide seasonal variation of the soil moisture régime at Yala, a more detailed analysis is necessary in order to evaluate the groundwater recharge. There are three well defined rainfall seasons at Yala, a $3\frac{1}{2}$ month wet season from mid-October to January, with a mean monthly rainfall of 6.4 in., a 4 month intermediate season from February to May, with a mean monthly rainfall of 2 in. and a dry season from June to mid-October, with a mean monthly rainfall of 1.2 in. The mean monthly free water evaporation being 4, 5 and 7 in. respectively during the above seasons, there is surplus water during the wet season, a moderate moisture deficit during the intermediate season and a severe moisture deficit during the dry season. Thus the water balance in the root zone fluctuates violently during the course of the year and groundwater recharge, therefore, cannot be a constant percentage of the annual rainfall.

In this study, the annual as well as the wet season rainfall, when analysed statistically, was found to be normally distributed. The variations of the different parameters of the hydrologic equation expressing the water balance of the root zone were computed, making certain necessary assumptions regarding water consumption by dry zone vegetation. The groundwater recharge which is the residual of rainfall was then computed.

It was found that the long-term mean annual groundwater recharge was approximately 8 in. There is a 16% probability that no recharge will occur in any one year and a $2\frac{1}{2}$% probability that no recharge will occur in two consecutive years.

SOUTHERN PART OF SRI LANKA SHOWING ANNUAL AVERAGE RAINFALL IN INCHES



Rainfall Distribution

Yala, situated in the south eastern sector of Sri Lanka is in the Dry Zone. The mean annual rainfall at Yala is 37.4 in., calculated for the period 1923 to 1971. The maximum and minimum recorded during this period were 62.9 in. and 21.6 in. respectively. The standard deviation was 9.1 in. (*Appendix* 1.) The values of rainfall **R** corresponding to a deviation of one standard deviation from the mean are 28.3 in. and 46.5 in. Of the 42 years of accepted annual totals, the rainfall was less than 28.3 in. during 8 years, between 28.3 in. and 46.5 in. during 26 years and greater than 46.5 in. during 8 years. These correspond to relative frequencies of 19 %, 62 % and 19 %. If the annual rainfall was normally distributed, the expected relative frequencies would be 16 %, 68 % and 16 %. The agreement seems reasonable, considering the fact that in a period as short as 42 years, the standard deviation derived from the sample distribution may deviate slightly from the corresponding statistics for the population. In order to test further the validity of the assumption that rainfall is normally distributed, the *Chi-Square test* was performed on the sample distribution. *Appendix* 3 gives the details of the computation. The observed and theoretically expected frequencies for different class intervals of **R** are shown in columns (7) and (6).

There is a marked seasonal variation of rainfall with the rainy season beginning in October and ending in January. The real dry season occurs during the Southwest Monsoon, mid-May to the end of September, while during the $3\frac{1}{2}$ months from February to mid-May the moisture conditions are intermediate between those of the dry and wet seasons. Further analysis of rainfall during the months of October and January by dividing each month into two halves shows that the wet season commences around the middle of October and ends at the end of January.

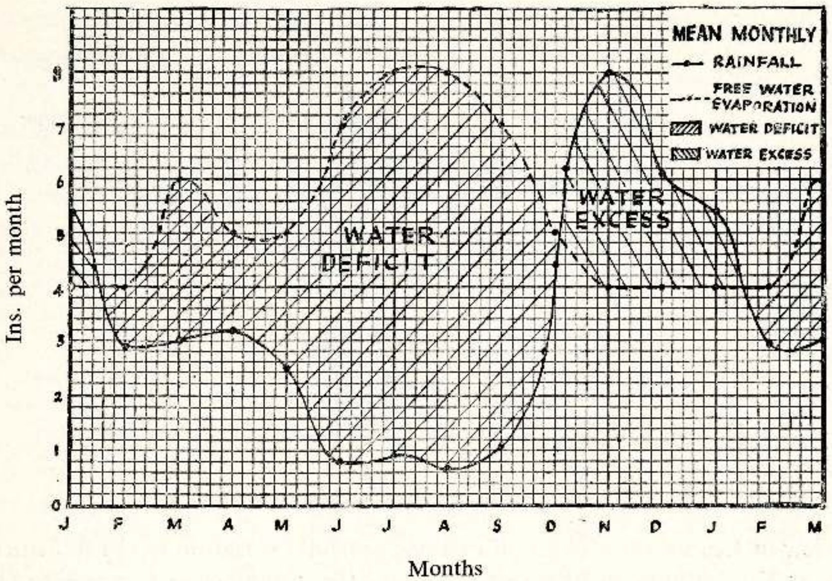


Figure 1. Mean monthly rainfall and free water evaporation at Yala

Mean Seasonal Moisture Balance

Figure 1 shows the mean monthly rainfall and assumed free water evaporation at Yala. In the absence of evaporation data at Yala itself, the adopted values are an interpolation of those at Ridiyagama and Inginiyagala and are in agreement with the saturation deficit variations at Hambantota and Batticaloa meteorological stations. The potential annual loss of water from a free water surface at Yala is approx. 67 in. while the mean rainfall is only 37 in., giving a moisture deficit of about 30 in. per year. However, due to the marked seasonal variation of rainfall, a moisture surplus of 8.3 in. exists during the wet season mid-October to end of January. This amounts to an average surplus of 2.3 in. per month during the wet season. During the intermediate period from February to May there is a total moisture deficit of 8.4 in. which amounts to an average of 2.1 in. per month. Very dry conditions prevail at Yala during the period June to end of September with a mean monthly rainfall of less than 1 in and a potential evaporation of over 7 in. The moisture deficit for this period up to mid-October is 27.6 in. and amounts to 6.1 in. per month. Thus the climate at Yala can be broadly divided into three seasons from the point of view of moisture availability.

Table 1

	Wet Season	Intermediate Season	Dry Season
Period	Mid-October to January	February to May	June to mid-October
Duration	3½ months	4 months	4½ months
Mean Rainfall per month	6.4 in.	2.0 in.	1.2 in.
Potential evaporation per month	4 in.	5 in.	7.5 in.
Moisture balance	Surplus 2.4 in. per month	Deficit 2.1 in. per month	Deficit 6.1 in. per month

In view of the above evidence of extreme seasonal variation in the moisture regime at Yala, it appears incorrect to assume the groundwater recharge to be either a constant percentage or the excess over a certain minimum of the total annual rainfall. If data of streamflow from a neighbouring catchment or the water levels of a well in the locality were available it would have been possible to assess the groundwater recharge by the normal hydrological methods. No such data is available for Yala. The method outlined below is approximate but in view of the lack of data required for more accurate analysis, the results may be of some value in estimating the availability of groundwater.

This investigation was primarily directed at estimating the groundwater recharge in the Maha Seelawa area within the Yala National Park. Since no well defined surface drainage system exists in the area, it may be assumed that no surface run off normally occurs and the infiltration capacity of the soil is greater than the rainfall intensities normally experienced.

Effective Rainfall

We may therefore assume that out of an amount of rainfall **R** received in a given period of time, all water, except that intercepted by all wetted surfaces including vegetation and held by surface tension forces, will infiltrate into the root zone of the soil whatever the rainfall intensity. The water held by wet surfaces is rapidly lost by evaporation at a rate greater than the rate of free water evaporation. For purposes of computation here, we assume this to be constant throughout the year and equal to 0.20 in. per day. The daily falls of less than 0.20 in. are thus ineffective in adding moisture to the soil and are lost by re-evaporation. The effective rainfall $\mathbf{R_e}$ in a given period can be expressed as :

$$\mathbf{R_e = R - (r + 0.2n)}$$

where **r** is the sum of all falls less than 0.20 in. and **n** is the number of days on which the rainfall was greater than 0.20 in. The gross, ineffective and effective total rainfall for the three seasons defined earlier and their monthly and daily means are given below:

Table 2

	Wet Season	Inter. Season	Dry Season
	in.	in.	in.
R	22.35	11.00	4.65
r	1.42	0.92	0.80
0.2n	5.20	3.20	2.40
r+0.2n	6.62	4.12	3.20
R_e	15.73	6.88	1.45
R_e per month	4.50	1.72	0.32
R_e per day	0.15	0.06	0.01

Infiltration

We now assume that all excess rainfall **R** enters the root zone of the soil through infiltration. While excess rainfall adds moisture to this store, evapo-transpiration losses cause depletion. This zone has a certain maximum water holding capacity S, determined by the soil type and the depth of the root system of vegetation. This represents the amount of water held in the root zone between the wilting point of vegetation and the field capacity of the soil. Any infiltration of rainwater occuring after this zone reaches field capacity, must pass beyond it into the deeper layers of the soil and finally into the groundwater reservoir. The root zone, therefore, regulates the flow of water into the groundwater reservoir so that an assessment of groundwater recharge is possible only through a detailed study of the water balance of this zone.

The water balance of the root zone layer during a given period of time can be expressed by the hydrologic equation :

$$\mathbf{R - r - E_T - I - \Delta S = 0.}$$

where **R**, **r**, $\mathbf{E_T}$ **I**, and Δ**S** are respectively, the gross rainfall, ineffective rainfall, evapo-transpiration, deep infiltration and increase in the soil moisture storage within the root zone. The above equation is valid over any period of time.

If we apply it for a complete climatic cycle (i.e. one year)

$$\Delta S = 0$$

Hence $\mathbf{I} = \mathbf{R_e} - \mathbf{E_T}$ *where* $\mathbf{R_e} = \mathbf{R} - \mathbf{r}$ is the effective rainfall.

Since the effective rainfall $\mathbf{R_e}$ is known, it would be possible to evaluate **I** if $\mathbf{E_T}$ were known. The problem therefore, reduces to the estimation of water consumption $\mathbf{E_T}$ by the vegetation.

Evapotranspiration from vegetation growing under saturated soil moisture conditions is proportional to free water evaporation $\mathbf{E_0}$

$$\text{i.e.}\quad \mathbf{E_T} = \mathbf{f.E_0}$$

where **f** is known as the crop factor and depends on the type and density of vegetation. *Fig.* 1 shows the mean monthly rainfall and free water evaporation $\mathbf{E_0}$ at Yala and may be considered to represent the water budget of a crop for which $\mathbf{f} = \mathbf{1}$. It is obvious that such a crop cannot survive here as the water deficit far exceeds the water surplus over the year. If the ordinates of the evaporation curve are halved, it lies below the rainfall curve for the period October through January to May and above the rainfall curve for the period May to October. This represents the water budget of a crop for which $\mathbf{f} = \mathbf{0.5}$ throughout the year. There is moisture deficiency for such a crop only during the period May to October. Under such conditions of limited soil moisture, evapotranspiration loss is a percentage of the soil moisture content. If we assume the root zone of the soil to be 3 ft. deep and the soil to have a moisture holding capacity of 1.3 in. per ft., this zone will have a field capacity of approximately 4 ft. Thus an average of approximately 1 in. of water is available per month during the dry season.

Appendix 4 gives the mean monthly values of the gross and effective rainfalls, free water evaporation and evapotranspiration loss for the finally adopted value of crop factor $\mathbf{f} = \mathbf{0.4}$. Rows **8, 9** and **10** give the derived values of $(\mathbf{R_e} - \mathbf{E_T})$, Δ **S** and **I.** Soil moisture depletion occurs from June to October and the amounts lost in successive months are 2.0, 1.0, 0.5 and 0.25 in. Soil moisture recharge occurs in the months October and November, the amounts being 1.8 and 2.0 in. respectively. The residual, groundwater recharge **I**, occurs in November, December and January. The amounts are 3.1, 3.2 and 2.3 in.

Thus in a year of average rainfall, out of the gross rainfall of 22.35 in. occurring during the $3\frac{1}{2}$ month wet season from mid-October to the end of January, an amount 6.62 in. is ineffective and is lost as re-evaporation. (*Table* 2) The balance 15.73 in. is effective in infiltrating into the root zone and beyond. Of this amount, 3.80 in is stored up in the root zone which had dried up during the preceding dry season and an amount 3.70 in. is consumed by vegetation for the evapotranspiration process during this period. The residual (15.73 - 7.50) = 8.23 in. represents the ground water recharge and must infiltrate beyond the root zone layer into the groundwater reservoir.

Variation of Groundwater Recharge

Since groundwater recharge is only a residual, in an average year when the gross rainfall is 22.35 in., its value is 8.23 in. In an year when the wet season gross rainfall is **R** in. groundwater recharge must be equal to **R**—14 in. approximately, since the net loss is 6.62 + 3.80 + 3.70 in. The wet season rainfall **R** shows wide variation from year to year and the data at Yala for a period of 47 years is shown in *Appendix* 3. Since this may be too short a period to be representative of the long term average pattern of variation, an attempt is made to fit the observed series to a theoretical statistical distribution. The *Chi-Square test* applied to the 47 year statistical series shows that **R** is normally distributed at least for the higher 90% of the values. The rainfall during the drier 10% of the years does not seem to follow the normal distribution, but this will not affect the results as no infiltration occurs during such dry years. Assuming normality, we deduce from the normal distribution, the rainfall values that are likely to be equalled or exceeded with stated probabilities in any given year (*Appendix* 6).

The results are given below

Prob.(P)	95%	90%	75%	50%	40%	30%	20%	10%	5%	2%
$\frac{R - \mu}{\sigma}$	-1.64	-1.28	-0.68	0	0.25	0.52	0.84	1.28	1.65	2.0
$R - \mu$	-13.6	-10.8	-5.6	0	2.1	4.3	7.0	10.0	13.7	16.6
Rainfall(R)	8.8	11.6	16.8	22.4	24.5	26.7	29.4	33.2	36.1	39.0
Groundwater recharge (I)	0	0	2.8	8.4	10.5	12.7	15.4	19.2	22.1	25.0

Where **R** = rainfall in in. μ = mean rainfall σ = standard deviation

Conclusion

The above investigation shows that the hydroclimate at Yala can be divided into 3 distinct seasons, from the point of view of the moisture balance of the soil. During the greater part of the year, the rainfall is insufficient to meet the moisture demands of evaporation and evapotranspiration. However, during the $3\frac{1}{2}$ month wet season from mid-October to January, rainfall is in excess of the evaporative demands and there is a surplus of moisture. Groundwater recharge, therefore, occurs only during the wet season.

It was found that the long-term mean annual groundwater recharge is approx. 8 in. or about 100 million gallons per sq. mile. There is a 84% probability that some recharge will occur in any given year, a 75% probability that this recharge would be greater than 3 in., a 50% probability that this recharge would be greater than 8 in. and a 25% probability that this recharge would be greater than 14 in. The probability that no recharge will occur in any one year is approx. 16%, in two consecutive years 2½%, while the risk that no recharge will occur in three consecutive years is ½%.

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Appendix 1

Annual Rainfall at Yala

Year	Total in In.	Deviation from Mean	Square of Deviation
1957	62.92	+25.53	651.60
1944	51.11	+13.75	189.10
1961	49.66	+12.27	150.50
1936	48.91	+ 11.52	132.70
1942	48.30	+10.91	119.10
1925	48.10	+10.71	114.80
1951	47.97	+10.58	111.90
1954	46.92	+ 9.53	98.15
1941	45.70	+ 8.31	69.05
1927	45.23	+ 7.79	60.67
1924	44.86	+ 7.47	55.80
1931	44.13	+ 6.74	45.43
1955	42.35	+ 4.96	24.60
1960	41.18	+ 3.77	14.21
1949	39.54	+ 2.17	4.71
1940	39.27	+ 1.88	3.53
1923	39.23	+ 1.84	3.38
1943	38.47	+ 1.08	1.16
1935	38.03	+ 0.64	0.41
1947	37.07	— 0.32	0.10
1926	36.98	— 0.41	0.17
1938	36.59	— 0.80	0.64
1933	36.34	— 1.05	1.10
1959	36.05	— 1.34	1.80
1953	35.70	— 1.65	2.72
1930	35.47	— 1.92	3.69
1971	35.40	— 1.99	3.96
1939	33.75	— 3.64	13.25
1946	33.40	— 3.99	15.92
1965	33.16	— 4.23	17.80
1962	29.98	— 7.41	54.91
1948	29.84	— 7.55	56.99
1937	28.89	— 8.50	72.24
1958	28.72	— 8.67	75.16
1956	28.24	— 9.15	83.71
1952	27.93	— 9.46	89.50
1928	27.88	— 9.51	90.44
1945	25.16	—12.23	149.60
1950	24.04	—13.35	178.20
1932	23.84	—13.55	183.50
1968	22.47	—14.92	222.60
1934	21.60	—15.79	249.40
Total	1570.38		3418.20
Mean	37.39		83.35

$\sigma^2 = 83.35$

Standard deviation $\sigma = 9.131$

Accepted annual rainfall totals have been arranged in descending order of magnitude. The mean annual rainfall of 37.39 in. is in agreement with the mean annual rainfall of 37.40 in. for the standard 30 year period (1931—60).

Appendix 2

Rainfall at Yala for the 3½ Month Wet Season Period
16 October to 31 January

Year	Total in Inches	Deviation from Mean	Square of Deviation
1957	51.60	+29.25	855.56
1951	38.76	+16.41	269.29
1931	37.30	+14.95	223.50
1929	32.15	+ 9.80	96.04
1936	31.90	+ 9.55	91.20
1969	31.62	+ 9.27	85.93
1923	29.76	+ 7.61	57.91
1963	29.74	+ 7.39	54.61
1941	29.73	+ 7.38	54.46
1961	28.16	+ 5.81	33.76
1933	28.00	+ 5.65	31.92
1955	26.58	+ 4.23	17.89
1966	26.15	+ 3.80	14.44
1944	25.96	+ 3.61	13.03
1940	25.77	+ 3.42	11.70
1959	25.26	+ 2.90	8.41
1948	25.03	+ 2.68	7.18
1926	24.74	+ 2.39	5.71
1924	23.69	+ 1.34	1.79
1954	23.65	+ 1.28	1.64
1942	22.50	+ 0.15	0.02
1935	22.32	— 0.03	0
1953	22.17	— 0.18	0.03
1943	21.66	— 0.69	0.48
1965	21.43	— 0.92	0.85
1925	21.33	— 1.02	1.04
1956	21.04	— 1.31	1.72
1937	20.09	— 2.26	5.11
1949	19.75	— 2.60	6.76
1960	19.51	— 2.84	8.07
1952	19.26	— 3.09	9.55
1971	18.56	— 3.79	14.36
1962	18.44	— 3.91	15.29
1938	18.31	— 4.04	16.33
1927	17.98	— 4.37	19.10
1946	17.90	— 4.45	19.80
1928	15.80	— 6.55	42.90
1939	14.02	— 8.33	69.39
1958	13.56	— 8.79	77.26
1950	13.14	— 9.21	84.82
1930	11.74	—10.61	112.57
1964	11.67	—10.68	114.06
1945	11.45	—10.90	118.81
1934	11.23	—11.12	123.65
1932	10.72	—11.63	135.26
1968	9.62	—12.73	162.05
1947	9.54	—12.81	164.10
Total	1050.26		3174.53
Mean	22.35		69.01
	Std. deviation	σ	— 8.32

Appendix 3

Chi-Square Test of Total Annual Rainfall — Yala

1 Order of Magnitude	2 Deviation from Mean	3 $\frac{R-R}{\sigma}$	4 Prob. (P)	5 1000–P	6 Expected Freq.e	7 Observed Freq.o	8 $\|e-o\|$	9 $\|e-o\|^2$
1	+25.53	2.8	997.4	2.6	0		—	—
5	+10.91	1.2	885	115	2.3	5	3	9
10	+ 7.79	0.855	805	195	8.4	10	2	4
15	+ 2.17	0.238	595	405	17	15	2	4
20	— 0.32	—0.035	488	512	21	20	1	1
25	— 1.65	—0.181	429	571	24	25	1	1
30	— 4.23	—0.462	323	677	28	30	2	4
35	— 9.15	—1.00	159	841	35	35	0	0
40	—13.55	—1.48	69	931	39	40	1	1
42	—15.79	—1.73	42	958	41		—	—
								24

$$\chi^2 = \frac{24}{5} = 4.8$$

No. of degrees of freedom $\nu = 8 - 3 = 5$

From χ^2 Tables, $\chi^2_{50\%} = 4.35$; $\chi^2_{90\%} = 1.61$

For $\nu = 5$, the value of $\chi^2 = 4.8$ corresponds to a probability of approximately 50% Hence departure is not significant *i.e.* assumption of normality is not incorrect.

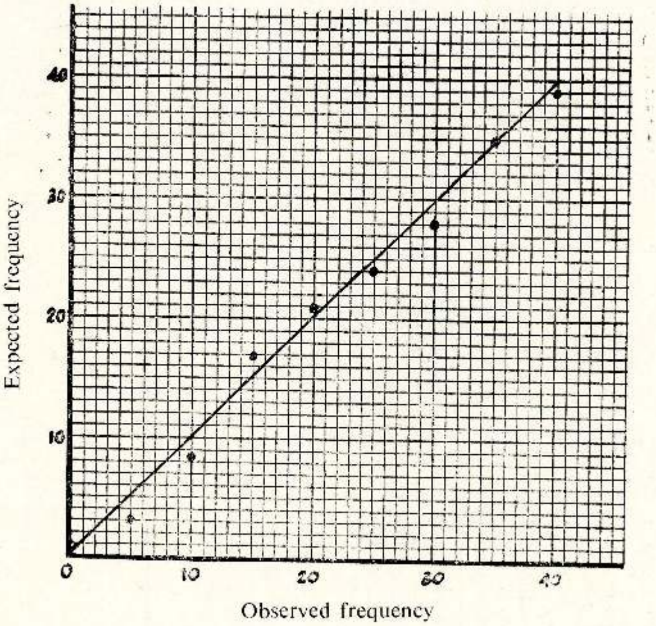
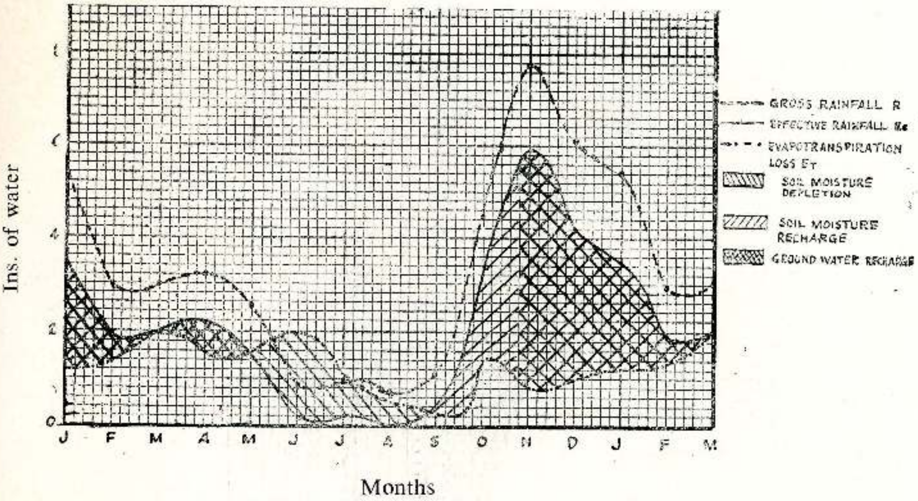


Figure 2. Observed vs expected frequency on assumption of normality

Appendix 4

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Gross rainfall (R) (in.)	5.4	2.9	3.0	3.2	2.5	0.8	0.9	0.7	1.1	4.4	7.8	6.1
Effective rainfall (R_e) (in.)	3.5	1.9	2.0	2.2	1.5	0.1	0.2	0	0.4	3.2	5.9	4.2
No. of raindays	8	5	5	6	6	3	2	3	3	9	14	11
No. of dry days (n)	23	23	26	24	25	27	29	28	27	22	16	20
E_o per day (in.)	0.13	0.14	0.19	0.17	0.16	0.23	0.26	0.26	0.23	0.16	0.13	0.13
$E_o n$ (in.)	3.0	3.2	4.9	4.1	4.0	6.2	7.5	7.3	6.2	3.5	2.1	2.6
$E_T = 0.4E_o n$ (in.)	1.2	1.3	2.0	1.6	1.6	2.5	3.0	2.9	2.5	1.4	0.8	1.0
$R_e - E_T$ (in.)	+2.3	+0.6	0	+0.6	−0.1	−2.4	−2.8	−2.9	−2.1	+1.8	+5.1	+3.2
$\triangle S$ (in.)	0	0	0	0	0	−2.0	−1.0	−0.5	−0.3	+1.8	+2.0	0
I (in.)	2.3	0.6	0	0.6	0	0	0	0	0	0	3.1	3.2


Ins. of water
8
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Months
GROSS RAINFALL R
EFFECTIVE RAINFALL Re
EVAPOTRANSPIRATION LOSS ET
SOIL MOISTURE DEPLETION
SOIL MOISTURE RECHARGE
GROUND WATER RECHARGE

Figure 3.

Appendix 5.

Chi-Square Test on Wet Season Rainfall — Yala

1 Order of Magnitude m	2 Deviation from Mean	3 $\frac{R-\bar{R}}{\sigma}$	4 Prob. (P) %	5 Expected Freq.(e)	6 Observed Freq. o	7 $\lvert e-o \rvert$	8 $\lvert e-o \rvert^2$
1	29.25	—	—	—	—	—	—
5	9.55	1.15	12.5	5.6	5	1	1
10	5.81	0.70	24.2	10.8	10	1	1
15	3.42	0.37	35.6	16	15	1	1
20	1.28	0.15	43.8	20	20	0	0
25	— 0.92	—0.11	54.4	25	25	0	0
30	— 2.84	—0.34	63.3	29	30	1	1
35	— 4.37	—0.53	70.2	32	35	3	9
40	— 9.21	—1.11	86.7	39	40	1	1
45	—11.63	—1.40	91.9	41	45	4	16
							30

$$\chi^2 = \frac{30}{5} = 6. \quad \text{for } m = 45$$

$$\chi^2 = \frac{14}{5} = 2.8 \quad \text{for } m = 40$$

From χ^2 Tables, $\nu = 9 - 3 = 6$

$$\chi^2_{50\%} = 5.35 \; ; \; \chi^2_{10\%} = 10.65$$

i.e. $\chi^2 = 6$ can be equalled or exceeded with a probability of about 45%. Hence departure is not significant *i.e.* assumption of normality is not incorrect.

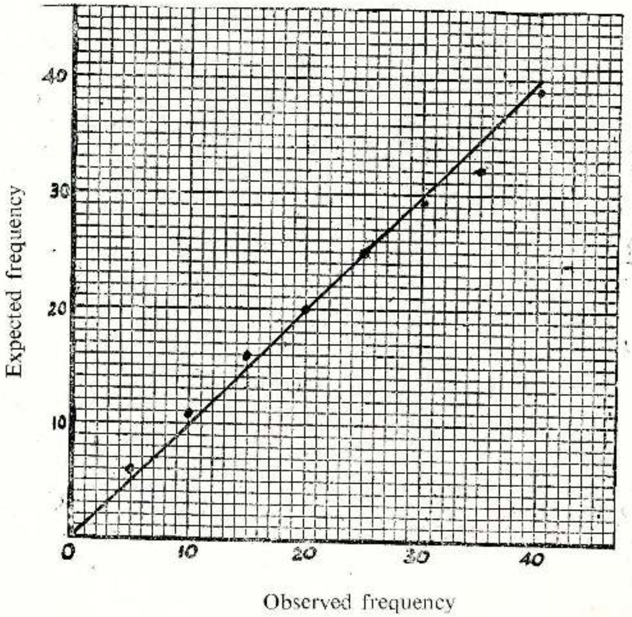

Figure 4. Observed vs expected frequency on the assumption of normality

Appendix 6.

Percent Probability(P)	95	90	75	50	40	30	20	10	5	2
Average Return Period(T) years			1⅓	2	2½	3⅓	5	10	20	50
Gross Rainfall (in.)	8.8	11.6	16.8	22.4	24.5	26.7	29.4	33.2	36.1	39.0
Groundwater recharge (in.)	0	0	2.8	8.4	10.5	12.7	15.4	19.2	22.1	25.0

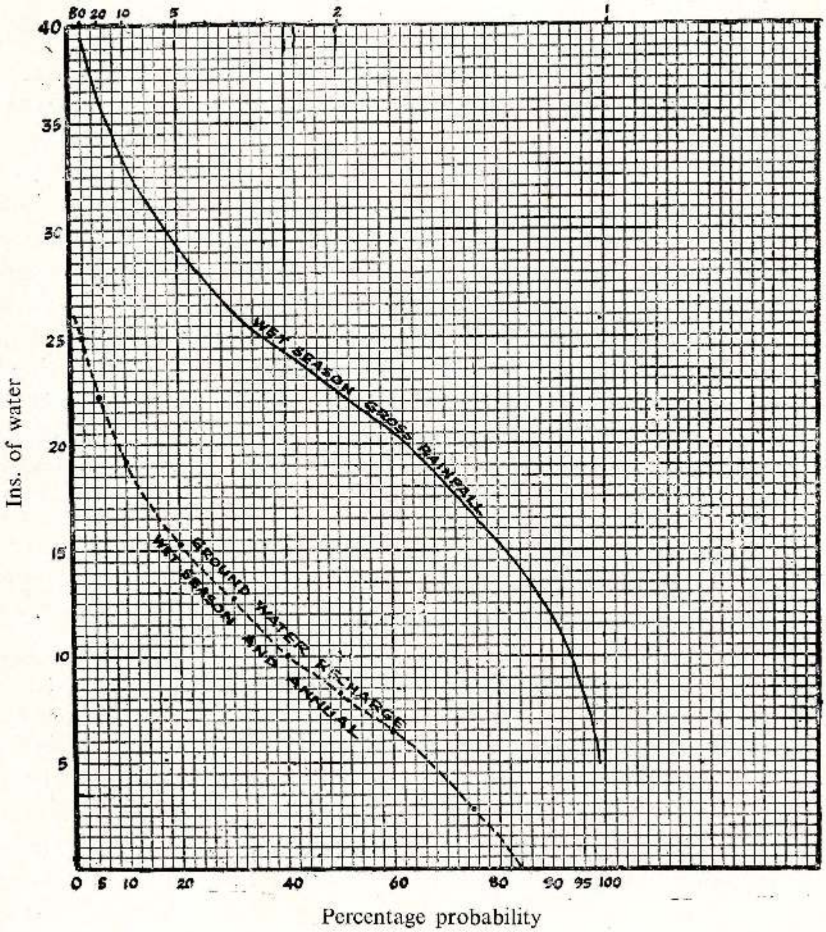

Figure 5. October to January — Yala

Rainfall and groundwater recharge likely to be equalled or exceeded with the stated probability in any one year

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Polysaccharides of Lichens

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It has been noted that lichens generally contain water-soluble polysaccharides in fairly high contents, but until recently, only lichenan, isolichenan and pustulan have been investigated chemically.

Lichenan (= lichenin) $[\alpha]_D + 9 \sim 18°$, $\overline{DP}$ 80 ~ 400, ir^{KBr}_{max} 890 cm^{-1} which was initially isolated from *Cetraria islandica* (Iceland moss) was studied extensively by several workers[2,11,12,15,26] and it has now been recognized as a linear homoglucan with $\beta(1 \rightarrow 3)$ $(1 \rightarrow 4)$ linkages in a ratio of 27 : 73 (3 : 7)[4]. The sequence of those linkages was studied by periodate degradation[6] and acid hydrolysis[21] obtaining glucose, laminaribiose (3-O-β-D-glucosyl glucose), cellobiose, cellotriose, 3 – O – β – cellobiosyl glucose, 4 – O – β – laminaribiosyl glucose, 4 – O – β – laminaribiosyl cellobiose, 3 – O – β – cellobiosyl cellobiose. Enzymatic hydrolysis of lichenan gave precise information about the sequence of linkages by the specificities of enzymes[23].

Cellulase of *Streptomyces* spp. QM B 814 which is known to split the $\beta(1 \rightarrow 4)$ linkage attached to a glucose unit whose 4—position is substituted, yielded 4 β – laminaribiosyl glucose as a main product, accompanied by 4 – O – β - laminaribiosyl cellobiose and 3 – O – β – cellobiosyl cellobiose, while laminarinase of *Rhizopus arrhizus* QM 1032 hydrolysing $\beta(1 \rightarrow 4)$ linkage of a 3 – substituted glucose unit afforded 3 – O – β – cellobiosyl glucose as a main product along with laminaribiose, 4 – O – β – laminaribiosyl cellobiose and 3 – O – β – cellobiosyl cellobiose. Neither laminaritriose nor branched disaccharide were found in the hydrolysate. The above results revealed that lichenan consists of tetrameric Glc $\beta 1 \rightarrow 3$ Glc $\beta 1 \rightarrow 4$ Glc $\beta 1 \rightarrow 4$ Glc as the main unit and pentameric Glc $\beta 1 \rightarrow 3$ Glc $\beta 1 \rightarrow 4$ Glc $\beta 1 \rightarrow 4$Glc $\beta 1 \rightarrow 4$ Glc as the sub unit.

Isolichenan (= isolichenin) $[\alpha]_D$ +255°, $\overline{DP}$ 34 ~ 43, ir^{KBr}_{max} 925, 845 800 cm^{-1} which occurs in lichens, being accompanied by lichenan, was shown to be a linear homoglucan with α (1 → 3) (1 → 4) linkages in a ratio of 57 : 43 (3 : 2). Isolichenan is more soluble in cold water than lichenan, and it is characterized by a slight blue colouration with iodine. The main part of isolichenan consists of Glc α 1 → 3 Glc α 1 → 3 Glc α 1 → 4 Glc linkages[7,22].

Earlier investigations showed the occurrence of lichenan and isolichenan in *Cetraria islandica, C. nivaris, Usnea barbata* and *U. longissima*[17]. Our recent investigations revealed the presence of both glucans in *Cetraria islandica* var. *orientalis, C. richardsonii, Parmelia tinctorum, P. conspersa, P. hypotrypella, P. nikkoensis, Alectoria sulcata, A. sarmentosa* and *Usnea bayleyi*.

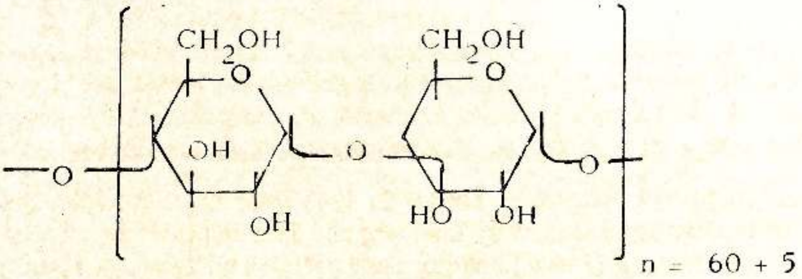
Pustulan was first isolated by Drake[5] in 1943 from *Lasallia pustulata* and *Umbilicaria hirsuta* and studied by Lindberg and McPherson[13] to formulate as linear glucan having β (1 → 6) linkage, since pustulan afforded on hydrolysis glucose, gentiobiose, gentiotriose, and gentiotetraose. This structure was also supported by the degradation of fully methylated pustulan[10]. According to our recent investigation, pustulan has been found characteristically in the lichens of Gyrophoraceae, e.g. *Gyrophora esculenta, Umbilicaria angulata, U. caroliniana, U. polyphylla*[20] and *Lasallia papulosa.*[24]. It has also been shown that pustulan contains 2% of O–acetyl group which is characterized by the ir absorption at 1735 and 1250 cm^{-1} and removed by alkaline hydrolysis with 2% aq. Na_2CO_3 at room temperature. The acetyl content in pustulan was determined by gas chromatography after hydrolysis, to reveal 1 acetyl group for every 10 to 12 glucose units.

By the method of Bouveng,[3] the native polysaccharide swollen in dimethylformamide was treated with phenylisocyanate to form a phenyl carbamate which was methylated by Kuhn's method and treated with $LiAlH_4$ to give a partially methylated glucan. The methyl ether in the 3-position of D-glucose was obtained by the hydrolysis of the partially methylated glucan to indicate, the acetyl group is attached to the 3-hydroxyl[19].

The molecular weight of pustulan has been determined by the equilibrium sedimentation method[33] to be c. 20 000 ($\overline{DP}$ c. 120).

PC–3 type glucan. *Parmelia caperata* contains a cold water insoluble polysaccharide tentatively named PC-3, and a soluble fraction (PC–2) which is identical with isolichenan. The polysaccharide PC–3 was methylated by Hakomori's method and the methyl ether was subjected to methanolysis to afford methyl 2, 3, 6 – tri – O – methyl *and* methyl 2, 4, 6 – tri – O – methyl – D – glucopyranosides, together with a small amount of methyl 2, 3, 4, 6 – tetra – O – methyl – D – gluco – pyranoside. Acetolysis of PC – 3 gave nigerose, maltose, 4 –O – α – nigerosyl D – glucose, and 3 – O – α – maltosyl D – glucose, whereas it gave neither maltotriose nor nigerotriose.

These results revealed that PC – 3 differs from isolichenan in the sequence of linkages and is rather similar to nigeran[1] an intracellular polysaccharide of *Aspergillus niger*, but the molecular size, $\overline{DP}$ 300 ~ 350, is bigger than the PC – 3 $\overline{DP}$ 100 ~ 130. Therefore, PC – 3 is formulated as follows:[31]



PC – 3 is contained in *Parmelia caperata, P. cetrarioides, P. laevior* and *P. saxatilis* along with isolichenan, and in *Cladonia bellidiflora, Cl. alpestris, Cl. pacifica, Cl. squamosa, Cl. crispata, and Cl. rangiferina* without co-occurrence of isolichenan.

Everniin. A cold-water insoluble polysaccharide, $[\alpha]_D + 138^\circ$, of *Evernia prunastri* was named everniin by Stude,[28] and studied by Mićović *and* Stojanović [16] and Stefanovich.[27]

The structure of everniin was formulated as a glucan having $\alpha(1 \rightarrow 3)$ $(1 \rightarrow 4)$ linkages in a ratio of 4 : 1 and molecular weight $1.8 \sim 3.4 \times 10^4$.

Our recent investigation showed that the polysaccharides of *Evernia prunastri*[30] are separated into three fractions, EP-3 (cold water-insoluble fraction), EP-6 and EP-7 (cold water-soluble fractions). EP-3, $[\alpha]_D + 200^\circ$, $\overline{DP}$ 70, is similar to everniin except in its specific rotation. Everniin might be a crude mixture of polysaccharides, since the earlier authors, in contrast to our recent results, isolated only everniin as the polysaccharide component of *Evernia prunastri*.

EP-6, $[\alpha]_D + 164^\circ$, $\overline{DP}$ 160, is an α-glucan having $(1 \rightarrow 3)$ $(1 \rightarrow 4)$, linkages in a ratio of 3 : 2, which is similar to isolichenan, $[\alpha]_D + 255^\circ$ $\overline{DP}$ 34 ~ 43, but it is slightly different in the molecular size.

EP-7, $[\alpha]_D + 12^\circ$, $\overline{DP}$ 60, is a β-glucan having $(1 \rightarrow 3)(1 \rightarrow 4)$ linkages in the ratio of 3 : 1, which is different from that of lichenan.

Acroscyphan. *Acroscyphus sphaerophoroides* which is a unique lichen growing in some very localized places of high altitudes along the Circum Pacific Region[25] contains, along with a glycopeptide a characteristic glucan for which I would like to propose the name, acroscyphan.

Acroscyphan, $[\alpha]_D + 176°$, ir 845 cm^{-1}, which is insoluble in cold water gives an intensive blue colouration with iodine. By the methanolysis of the methyl ether, methyl 2, 3, 4, - tri - O - methyl-, methyl 2, 4, 6, - tri - O - methyl—*and* methyl 2, 3, 6 - tri - O - methyl - D - glucopyranosides were afforded along with methyl 2, 3, 4, 6-tetra - O - methyl - D - glucopyranoside.

It has therefore been revealed that acroscyphan is a homoglucan having $\alpha(1 \rightarrow 3)(1 \rightarrow 4)$ $(1 \rightarrow 6)$ linkages[30].

An $\alpha(1 \rightarrow 3)$ $(1 \rightarrow 4)$ glucan (1 : 4) was isolated from *Stereocaulon paschale*[9] Our recent investigation showed that a similar glucan is also contained in *Stereocaulon japonicum* and *St. vesvianum*. A survey work on the polysaccharides of *Stereocaulon* spp. is now under progress.

Glycopeptides are distributed widely in the lichens of *Lobaria* spp. and *Sticta* spp. without co-occurrence of simple glycans[29].

The following lichens were studied in order to find the occurrence of glycopeptides which accompany PC-3 as minor components.

Stictaceae: *Lobaria orientalis, L. isidiosa, L. pseudopulmonaria, L. linita, L. japonica, Sticta gracilis, S. wrightii ;*

Cladoniaceae: *Cladonia bellidiflora, Cl. alpestris, Cl. pacifica, Cl. graciliformis,* (*Cl. squamosa, Cl. crispata* and *Cl. rangiferina*)

The glycopeptides of *Lobaria orientalis* were separated into LOF-1, $[\alpha]_D + 50°$ ir_{max} 875 cm^{-1}, N, 1.01 %, and LOF-2, $[\alpha]_D + 31.5°$, ir_{max} 875 cm^{-1}, N, 1.04 %.

The sugar components of LOF-1 determined by a sugar analyzer were glucose and galactose as the main components and mannose, arabinose, xylose and rhamnose as the minor components. LOF-2 contains mainly mannose and galactose.

After treatment of LOF-1 with alkali followed by reduction with $NaBH_4$, the reaction mixture was analysed using an amino acid analyzer. In contrast to the acid hydrolysate of LOF-1, a decrease of serine and threonine which corresponded to the increase of alanine and α-aminobutyric acid was observed by the above treatment.

Thus the carbohydrate portion of LOF-1 is linked with the peptide part by an O—glycosyl linkage with serine and threonine. The methanolysis of permethylates of LOF-1 indicated that $1 \rightarrow 6$ glucan and $1 \rightarrow 3$ mannan are the main components of its carbohydrate moiety.

By the results of our survey a wide distribution of various homo-and hetero-glucans in lichens has been demonstrated to suggest their chemotaxonomic characters.

The lichen metabolities of smaller molecular weights are specific species while polysaccharides are generally recognized as being characteristic for a larger group, such as a Family or Genus, as shown in the occurrence of pustulan in Gyrophoraceous lichens. Our experiments of laboratory cultivation of the separated mycobionts of *Ramalina* spp. showed that the lichen polysaccharides might mostly be produced by the mycobionts, and the polysaccharides of phycobionts gave a different character in comparison with those of intact lichens.

One of the most characteristic biological activites of most lichen polysaccharides is their host-mediated antitumour actions[8]. It has almost been established that some polysaccharides of fungi and lichens could inhibit the growth of implanted sarcoma 180 in mice, by the *i.p.* injection once a day during 10 days period to the animal, 24 hr after the implantation of the tumour cells. The results are observed after 5 weeks, measuring the inhibition ratios and the rate of complete regression of tumour in comparision with the control[18].

In general, homoglucans with $\beta(1\rightarrow3)$ $(1\rightarrow4)$ or $\beta(1\rightarrow6)$ linkages are more effective than α-glucans. The molecular size and some fine structures would also be related to the biological activities, since oat-lichenan, dextran of various molecular weights and islandic acid are inactive against the tumour growth.

The mechanism of this host-mediated antitumour activities of fungal and lichen polysaccharides has not yet been established. According to Tokuzen[32], the antitumour active polysaccharides *i.p.* injected to the animal caused extensive outpouring of lymphoid cells, plasma cells and macrophage in the vicinity of the graft about 1 week after implantation of sarcoma 180 in mice, and later invasion of connective tissue cells into the graft about a week after the implantation of tumour cells. The control experiments using the antitumour inactive polysaccharides showed no such characteristic cell action. The polysaccharides having potent suppressing effects against allogenic tumour failed to give suppressive effects against autochotonous tumour. Maeda and Chihara[14] also studied the mechanism of antitumour effects of fungal polysaccharides such as lentinan of *Lentinus edodes*, and concluded that lentinan is an immunopotentiator, and stated that a sensitizing state against serotonine or histamine would have some correlation with the antitumour activity of such polysaccharides.

Acknowledgements

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An Experiment with the Bilingual Method for Teaching English as a Complementary Language

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Abstract The purpose of the experiment was to see whether the use of a bilingual technique would result in improvement in English. The method was to measure imitation response of pupils both before and after the use of the technique. The control groups were pupils who followed the usual classroom method in vogue in Sri Lanka (Ceylon) today. Pupils were mainly Grade Nine pupils, categorized into *B*, *A* and A + groups on the basis of their parents' income level, occupation and cultural level.

The lesson material was organized into three units, consisting of : (*a*) Short passages in the mother tongue which provided "cues" for sentence patterns to be taught and served to define and limit the structures to be learned by the pupils ; (*b*) Comprehension passages correlated with (a) in sentence patterns and structural words only ; (*c*) Drills for practising the patterns used in (a) and (b).

The initial and final tests sampled formal areas of language, comprehension and free composition, the final test being more difficult because of the test of "recall" rather than "recognition" knowledge. All categories of pupils (*B*, *A* and *A* +) showed a definite improvement, regardless of teacher competence.

1. *Introduction*

1.1 Pedagogically an English lesson is analysable into two activities: (a) an attempt to convey meaning, and (b) an attempt to produce imitation.[2] It may be viewed from the angle of the learner as an attempt to *acquire* and *retain* meaning and at the same time to perfect an imitation response. There is no need to haggle over whether the imitation response is to be a spoken imitation or a written imitation. Even if our aim is to obtain written imitation, it is doubtful whether it would be possible without some measure of vocalization.*[5,8]

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* "……internal speech may be carried out wholly by processes within the nervous system, with some unessential discharge upon the final common path for vocal movements "[5]

1.2 There is the view that English is learnt for reading--comprehension only, which some naively take to mean that no kind of imitation response, whether in speech or writing, is necessary. It is being assumed in this paper that even the reading—comprehension aim is not possible of attainment without at least one category of imitation response, namely vocalization. Some form of vocalization is found to be necessary even in the teaching of dead languages and in the use of the grammar translation method as well.**[6] Thus for this paper it is assumed that spoken imitation is necessary to some extent in the learning of an additional language if not as an end itself as a technique of assimilation.[11]

2. *The Constituents of Method*

2.1 A method of teaching a language involves four aspects : (a) selection (b) grading (c) presentation (d) repetition.[7]

2.11 No one ever teaches the entire body of knowledge relating to a subject, a **selection** is made of the content. For example, if the course is for tourists, words may have to be selected with a social and commercial bias. Selection will be conditioned not only *by the function* of the language, but also *by the age-level* of the learners ; *by the similarities and dissimilarities* between the mother-tongue and the additional language ; *by the particular variety* of the language that is being taught, there being in the case of English, among other varieties, Canadian, General American, Received English and even Ceylon English [constituting the *dialect*] ; *by the social group* whose language is being taught, for *technical* English and *society* English will be different in themselves [constituting the *register*]; by whether the language is to be taught primarily for a *reading--comprehension use*, or for a *conversational ability* [medium] ; and by whether the language is that of *an educated class*, *a strictly professional sub-class*, or of *an international class* of academic users of the language [*style*].

2.12 **Grading** will be conditioned by one's conception of the central characteristics of the language, and it will ultimately depend on one's assumptions about language. If one uses a *referential* description of language, one's grading will be in meaning units ; if a *differential* description of language, on sound units, involving identification of phonemes, allophones, monophones, and the variety of sound features and sub-features that have been described by linguists.

2.13 **Presentation** is that aspect of method which concerns this enquiry. It is the only aspect of method which involves *inter-personal relations*. Any

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**Jean Comenius used imitation, repetition and plenty of practice in both reading and speaking.[6]

consideration of this aspect shifts the focus from the study directly into the classroom, into the dynamics of classroom activity. Both *presentation* and the next aspect of method, *repetition*, which is the fourth, focus on class-room activity.

Presentation has two sub-aspects : (a) linguistic and (b) technical.

(*a*) The *linguistic aspect* consists of whether presentation should be spoken or written and what proportion of speech and written work should be used ; whether situations and contexts should be used for words, or whether word lists alone would do.

(*b*) The *technical aspect* has to do with what aids and devices should be used for meaning-conveying : whether the mother tongue together with or without pictures, actions etc. should be used, or only audio-visual, and action based devices; whether real or artificial situations, pictures should be used or whether the mother tongue alone should be used. It takes up the question of the degree to which there should be the use of gadgetry, such as audio-visual aids, films, language laboratories, or such home-made contraptions as puppets and flannel boards.

2.14 **Repetition** deals with questions such as how what has been taught may be kept up, and the extent to which in this process, speaking, reading and writing should figure. It is a question of the means for habituating and consolidating what has been presented.

3. *The Aim of the Experiment*

3.1 The experiment was to examine the success of a bilingual technique of presentation of an English lesson for meaning-conveying. This was assessed by measuring initially the written imitation response of pupils in English, and after use of the bilingual technique, by measuring imitation response again. It has already been established that successful meaning-conveying would result in successful imitation response.[3]

4. *Attendant Difficulties*

4.1 The experiment which was to have been of a year's duration was reduced to a two term period because of political conditions in 1971. A further disadvantage was that the teachers who were diploma students handed over the teaching to the class teacher in the course of the experiment. Yet the duration was sufficient to arrive at some clear conclusions regarding the effectiveness of the technique. There was an additional reason for the inability to work the course as effectively as one would have liked. Teachers seemed obsessed with the idea that what had to be done in class was — regardless of the difficulty of the texts — the reading of the government texts, failure to do which might result in a reprimand. Though it was pointed out that this Course being tried out for teaching English would result in perhaps better understanding of the texts, the argument cut no ice. Teachers wanted permission to return as fast as possible to the reading of the texts, regardless of whether pupils were capable of making anything at all of them. *In short it means that for a long-term experiment the University must have its own school where freedom to experiment would be unhindered, or the Ministry of Education would have to re-assure teachers about co-operation in research.*

5. *The Classes and the Schools*

5.1 The classes involved were Ninth Grades mainly, though teachers were permitted to try out the lessons in Grades 8 as well as 10. Where the lessons were tried out in Grade 10 systematically, the results have been taken into account for the findings. The streams of Grade 9 that were not involved in the experiment were the control groups.

5.2 The classes were from schools with children of differing background :-

B Category of School	Girls High School *Grade 9*	32 pupils (*Tamil Medium*)	English literate home background
	Mowbray Girls School *Grade 8*	37 pupils (*Tamil Medium*)	-do-
	Mahamaya Girls School *Grade 8B*	32 pupils (*Sinhala Medium*)	-do-
A Category of School	Getambe Senior School *Grade 9*	32 pupils (*Sinhala Medium*)	Sinhala Literate home background
	Hindagala Maha Vidyalaya *Grade 9*	33 pupils (*Sinhala Medium*)	-do-
	Kandy Hindu Senior School *Grade 9*	32 pupils (*Tamil Medium*)	Tamil Literate home background
A + Category of School	Govt. Tamil School, Peradeniya *Grade 9*	14 pupils (*Tamil Medium*)	Non Literate background

Sri Rahula, Katugastota, also came into the experiment but because of the ill-health of the teacher could not complete the experiment. Mahamaya was not able to go on with the project because of the already--mentioned stranglehold of the government texts.

5.3 The classes were differentiated mainly on the basis of their home background, but such factors as physical facilities available in the school were also taken into account. Categories *B, A* & *A* + were used to describe the better, average and below average classes in relation to the different background and learning opportunities.

6. *Basis of Categorization*[10]

6.1 The categorization was in relation to certain tangible factors which may be important for planning English courses in developing countries :

(*a*) the income level
(*b*) the occupation of the parents
(*c*) the literacy of the parents
(*d*) the cultural level

6.2 For '**B**' **category** of class — the income of the majority of pupils' parents was between Rs. 400/- & 700/- per month. At Mahamaya, the Grade Nine class was made up of 65.6% of students whose parents had a monthly income of over Rs. 400/-. At Mowbray more than 50% of the pupils' parents had an income of between Rs. 400/- & 700/-. At Girls High School 59.5% of the pupils were from homes enjoying an income of over Rs. 400/- per month.

In all these three instances of Grade Nine the literacy rate was high. There certainly was a positive correlation between the income levels of these groups (which ranged from Rs. 400 upwards) on the one hand and mother-tongue as well as English literacy on the other. At Mahamaya, mother-tongue literacy was 100% — both parents being literate. English literacy was 34.58%, the criterion being both parents' ability to read and write English. English literacy in terms of a single parent's ability to read and write English was 66%.

At Girls' High School there was a similar correlation. 84% of children claimed that at least one parent (generally the father) was conversant with English. 70% of the pupils claimed English was used occasionally at home.

At Mowbray again it was 100% mother tongue literacy and a high percentage of English-familiarity among parents.

6.3 Thus the *criteria* of '*B*' category were (a) an over 50% group in the class enjoying an income of over Rs. 400/- a month. (b) An over 75% mother tongue literacy at home. (c) An over 50% literacy in English at least in the case of single parent literacy.

6.4 '**A**' **category** of pupil represents the class of society which should be the main concern of the State in its English teaching programme. It constitutes by far the largest number of the school population. The higher income group of the '*B*' category will often be able to find ways and means of improving English, and will learn English however inadequate the official programme and its methodology are. These pupils will often get home instruction in English. "*A*" category of pupil learns no English at home, and his home background is one of pure mother-tongue literacy, though it is basic literacy—

most fathers not having had more than a primary education. By parental literacy is meant mainly the father's literacy. In some cases both parents were literate in the mother tongue. The income range was below Rs. 200/- per month. The occupation of parents was cultivation (agriculture), or self-employment as carpenters or masons. Often both parents worked.

Cultural and religious pursuits mean only reading the newspaper if it is available and generally by one parent only (whose reading speed cannot be high) and a temple visit on a full-moon day. Inspite of mother tongue literacy, not one home possessed a Buddhist *Jataka Potha* or *Bana Potha.*

At the Hindagala school more than two thirds of the pupils in Grades 9C and 10A were from homes in which the parents earned Rs. 175/- on an average a month. The average family size was seven. Over 60% of the parents were literate in the mother tongue. Only 5% of the families could boast of both parents being literate. Only one parent was literate in English.

At the Hindu Senior School the average income was Rs. 168.13 and over 50% in class were from homes in which both parents together earn less than Rs. 200/-. Over 60% of the parents were literate in the mother tongue, Tamil. Only 2 parents had a knowledge of English.

At Getambe School the family income of more than 50% of the parents was less than Rs. 200/-. More than 60% of both parents read and write Sinhala, the situation here being better than in the earlier two schools, but more than 90% of the literate parents have had only a primary education. Just three parents read and write English.

6.5 The application of our criteria in the case of these three schools shows a marked difference in (a) income & (c) English literacy from the earlier schools in which our '*B*' Category pupils were found and less markedly in regard to (b) which is mother tongue literacy. Thus in objective, measureable terms there is a clear difference between *A* and *B* category pupil-grades.

6.6 **A + category** of pupil is equally clearly demarcated. Parents in the Govt. Tamil Senior School, Peradeniya *were said to have* an average income of Rs. 123/-. But when further enquiries were made, one doubted whether the average income of Rs. 123/- could be correct. One boy claimed that his father was employed in a firm in Kandy and that he lived in a four-roomed house. But on a visit by the teacher to his home, it was discovered that his house was a one-roomed hut, and that his father was unemployed. Most of the parents, on investigation, were found to be casual labourers or estate employees. Thus it seems likely that, the average income of parents was less than the proferred figure of Rs. 123/-.

As for *literacy* level, again results of investigations belied the claims which were that all could read and write. Some parents could not read a newspaper and others could only write their names. None of the parents knew English.

More than 60% of the parents were unskilled labourers or estate coolies. They lived in Hendeniya, Udapolawatte, Pottapitiya, Gannoruwa and Godakandeniya.

6.7 The *facilities* available in the schools also have some relation to the categories of *B*, *A* & *A*+. The category *B* classes were in schools which had facilities — better buildings, library and laboratory facilities, furniture, playground space etc., whereas:

> At Hindagala "the principal's office, three halls, four dilapidated latrines, a few almirahs—two with soiled library books which are hardly given out, a couple of mammoties and a pile of broken furniture speak for the facilities the school enjoys. Perhaps the small plot of land used as a playground completes the picture."[4] The school has a long and congested hall with 12 noisy classes crammed together without partitions. The teacher describes the school as "just a masonry structure which shelters the teacher and learner for a few hours".[4]
>
> At Getambe the pupils in the selected grades for the experiment had no class room but some old partition boards gave it a " semblance of a separate class room. Furniture was inadequate and some pupils had to share the furniture. Floor space was restricted, so that the class was tightly packed with desks and chairs. The roof of the school building was in a state of disrepair so that when it rained no work could be done ".[1]
>
> The Hindu Senior School, Kandy, likewise suffers from an acute lack of many essential facilities. The school building consists of only two halls. There are no separate class rooms. The two halls are converted into classrooms which are separated only partly by small screens. Even the toilet facilities are sub-standard.
>
> Facilities in the *A*+ school are several degrees worse. The Govt. Tamil School, Peradeniya is a Kanishta Vidyalaya (Junior School). It does not have adequate furniture, teaching aids, space or even proper sanitary facilities. There are no class rooms, no screens even and the entire hall looks like a wattle and daub structure disguised by means of a coat of limewash. It is a half walled structure with gaping holes in the roof.

7. *The Experiment*

The lesson material was of three types:

(*a*) English passages with Sinhalese or Tamil equivalent for reconstruction by the pupils. The purpose of the mother-tongue equivalents was for providing cues for the construction orally of the English passages. Pupils did not see the English passages during the lesson until they had themselves reconstructed them orally and then in writing. These English passages were called "*Reconstruction passages*".

(*b*) *Comprehension passages* correlated with (*a*) in regard to sentence patterns and structural words only.

(*c*) *Drills* for practising the patterns and structural words in (a) & (b).

The lesson material was what had earlier been used in pilot projects in 1967 & 1968 before the printing of the Grade Nine Book based on the bilingual technique of reconstruction which was later withdrawn. The material had been arranged as weekly units in the original book, with a theme for a week. The first week was devoted to two passages on a *farmer* and a *smith*. The next week's passage was entitled "*The farmer works hard*" and "*The farmer in harvesting time*". This theme continued and varied in week 3 as "*The farmer, the villager and the harvest*". The repetition was to provide consolidation, and the slight change in content was to sustain interest. In week five the theme moved away from the farmer though it still centred round the rural environment to "*The Sinhalese New Year*" and from here one worked on to *Festivals* in the same week and in week 6 the interest was focussed on the specific festivals of *Vesak* & *Poson*. Thus the material was organized in relation to themes which were sometimes repeated under varied conditions, but always set realistically in the environment of the pupils. There were no passages on picnics to the sea or games of cricket or on what serendipity means, which are some of the topics of the government textbooks and radio English programmes.

The next observation about the material was that repetition was built into the material through exercises involving construction of English sentences through mother tongue cues. No use was made of substitution tables ; there were however some jumbled-sentence exercises, and filling in of blanks with again mother tongue cues to help.

8. *The Mode of Administration*

8.1 The administration of the material was preceded in every case by a test to check on present attainment (*imitation response*). In several instances this attainment test which was kept confidential was re-administered after the end of the series of lessons, together with another achievement test geared specifically to the content of the course being tried out to find out how much had been assimilated. In certain instances an intelligence test, verbal (*Jayasuriya*) and non-verbal (*Raven*), was administered by the teacher on his own initiative to satisfy himself about the I.Q. of his class.

8.2 The mother tongue version of the passage for reconstruction was first administered, and then after several oral attempts had been made at reconstruction, was reconstructed in writing by the pupils. After correction by the teacher of the written exercises, the English passage which is the teacher's model passage was distributed, read orally by the pupils, used for comprehension as far as possible and written out, even as transcription. This took two or three lessons. At the fourth lesson the comprehension passage was done in class and the exercises that followed were answered both orally and in written

English. This again took at least two lessons. After the corrections had been made the drills were taken up and again at least two lessons were devoted to this. Thus as many as seven lessons were necessary for a week's unit, and a week's unit did often run on into the next week, as generally a week's English is only five lessons. However the order of lessons for each set of seven lessons was (a) an initial reconstruction lesson followed by (b) a comprehension and (c) a drill lesson.

8.3 Every reconstruction lesson used the mother tongue for meaning-conveying. The lessons that followed the reconstruction lesson used relatively less mother tongue. It was observed that at the beginning the quantum of mother tongue was heavy but as the lessons proceeded less and less of the mother tongue needed to be used, and the pupils' response to instructions and questions quickened showing that once meaning-conveying had effectively taken place through the bilingual method, it ceased to be a problem requiring special solution.

9. *Evaluation Tests*

9.1 The following tests were administered : an attainment test at the beginning and the same test at the end of a specific period of teaching. This test was not in any way connected with the content vocabulary of the lessons. Another test of greater difficulty was administered.

9.11 **Test I.** The attainment test contained 50 test items, of which only 5 involved *recognition* of the correct answer from a choice of answers. This is an easier skill to acquire than *recall* knowledge which involves recalling the correct answer without the help of an available series of choices. These 5 recognition test items were to test the ability to recognize the meanings of English vocabulary.

38 test items tested the more difficult skill, namely the ability to *recall*. The recall started with the *alphabet*, as the test had a diagnostic character. The other formal areas of language tested were *spelling*, the *sounds*, *vocabulary*, *grammatical mechanisms* such as formation of plurals etc., *word order of sentences*, *prepositions*, *articles* and other *miscellaneous parts of speech*.

3 items were a test of the comprehension skill of what for convenience may be referred to as the *identification of factual or textual matter*, i.e. the skill does not involve writing out of a sentence of one's own after reading a passage, but only reproducing a phrase or word from the actual text, as the answer to the comprehension question does not involve anything more than an identification of the words in the text which contain the answer. Thus the comprehension was of the easiest kind.

The writing of four sentences (called *free composition*) was required. Thus there were 4 free composition items.

This was the test used in all the schools just prior to the teaching experiment except in the case of the Hindu Senior School, Kandy, where the teacher constructed his own test though on the same specifications. For example, this latter test gave marks as follows : 8 marks for recognition items, 61 for recall, 9 for comprehension and 22 for application, whereas the earlier test had the following scheme of marks ; 5 marks for recognition, 51 for recall, 7 for comprehension and 16 for composition. Thus the proportions were generally alike.

9.12 **Test II.** A further test of greater difficulty was administered after the course of teaching was over. It contained not a single test item for the easier *recognition* knowledge but there were 25 items for *recall*. There were 5 items for *comprehension* but of these only 2 questions were a test of the **I.M.** variety (*identification of factual matter*). Two questions involved writing out a new sentence in answer to the comprehension question — a more difficult skill. These questions we may designate *understanding of relationships* (**U.R.**). One question was more difficult than an I.M. question but not as difficult as a U.R. question and was designated an **I.M.*** (I.M. starred).

Finally there were 10 *composition items*. There was a total of 40 items as compared to 50 in the earlier test, but the 40 items were of an infinitely harder variety. 25 items were recall. Whereas in test I there were only 4 compositional items and 3 comprehension items, in test II there were 10 compositional items and 5 comprehension items. Knowledge of grammatical structure was tested only by 18 items in test I, whereas this test sampled the area of grammatical structure with 25 items. In one respect it could be considered easier than test I, namely in that *except for the comprehension questicns* it used a vocabulary content which echoed the vocabulary content of the teaching material. The findings of this paper are not based only on improved performances in this test, for as stated, test I was again re-administered to the pupils before test II was used. Both tests are analyzed on page 199 and the table of specifications is available in *Appendix I*.

10. *Comparison of test performance*

10.1 In every instance, whether of category *B*, *A* or *A+*, pupils had distinctly improved in their performance after the course of teaching.

10.2 Tabulation of arithmetic mean (A.M.) and standard deviation (S. D.) results for the different schools are as follows:

Category	*School*	*Test I*	*Test II*
B	Girls' High School	54.28 (S.D. 12)	85.18 (S.D. 14.7)
	Mahamaya	46.2 (S.D. 18.27)	50.7 (S.D. 24.48)
	Mowbray	47.5 (S.D. 1.8.)	54.2 (S.D. 14)
A	Getambe	37.8 (S.D. 13.5)	43.3 (S.D. 17.9)
	Hindagala M.V. (*Grade* 9)	30.9 (S.D. 7.8)	70.73 (S.D. 15.7)
	Hindagala M.V. (*Grade* 10)	23.6 (S.D. 8.1)	54.6 (S.D. 14.7)
	K/Hindu Senior	36.0 (S.D. 11.10)	52.8 (S.D. 19.75)
A+	Peradeniya Govt. Tamil School	32 (S.D. 11.1)	41 (S.D. 21.4)

10.3 The difference in improvement ranges between 6% to 30%. One reason could be the difference in personality factors of the teachers. For example regarding the high rate of improvement at Girls' High School where the A.M. for the 1st test was 54.28 and for the final test was 85.18, the teacher concerned received some of the highest grades in the diploma in education examination, and obtained a merit pass. At Hindagala (a school which has been categorized *A*) the improvement was even better—the mean for Test No. I being 30.9 and for Test No. II, 70.73. This teacher too had scored an excellent grade in his practical teaching examination for the diploma in education. Thus the rate of improvement differs according to personality factors of the teachers. The point however is that improvement took place *in every case*, regardless of who the teacher was.

10.4 Other reasons could be (a) I.Q. differences among students, and (b) facilities in schools, etc. For example the very good improvement registered at Girls' High School where high income group parents send their children is paralleled by a high rate of improvement at Hindagala where low income group parents send their children. The reason may be that I.Q. is also a contributory factor. Both verbal (*Jayasuriya*) and non-verbal (*Raven*) I.Q. tests revealed at Hindagala an A.M. of 99.66 (for the verbal test) and 99.9 (for the non verbal test). I.Q. seems to have a say from class to class as well, for the I.Q. for Grade 10 of the same school was 83.5 (verbal) and 94.2 (non verbal), which may account for the lower A.M. at Test II in Grade 10 though the rate of improvement was about the same. The same teacher took both classes. The A.M. for Grade 10 at Hindagala was 54.6 as against 70.73 for Grade 9 of the same school.

10.5 What is important is that all students registered an improvment. *This means that the use of the mother tongue for meaning conveying is not dependent for success on high grade teacher qualities.* Any average teacher car produce good results through it though it is inevitable that high grade teacher qualities could made a difference anywhere in the world to the degree of success of any methodology. This is an important condition in favour of a methodology in a country in which competence in English among teachers is fast declining, and in which centralizing of direction is affecting the teacher's freedom and initiative, two requirements that appear to be needed for fostering high grade teacher qualities. A technique using direct method devices of meaning-conveying draws heavily for its success on high grade teaching, and often fares miserably in the hands of average or incompetent teachers. *This is not a draw-back of the reconstruction technique which uses the mother tongue for meaning-conveying.*

11. *Other Observations*

11.1 Some other interesting observations relate to the re-administering of test I after use of the material, before the administration of the second test.

At Hindagala for instance in the second administration of test I, A.M. rose to 46.3 from 30.9, an increase of nearly 16. The teacher wrote :

> " The Grade 9C A.M. rose from 30.9 to 46.3. The number of items omitted showed a marked decline and in one group of items it entirely disappeared. The number of correct responses more than doubled ".

For example in the first administration, word order questions were all wrong, but in the second administration 50% of the answers were correct. With regard to comprehension and composition in the 1st administration there were 17% of correct answers but in the second administration the percentage rose up to 45.4%. The teacher continued :

> " However the dispersion in the re-test was wide, ranging from 22 to 58 with a S.D. of 10.7. This may perhaps be due to the attention paid to individual children who could proceed at a rapid rate or else may be due to the encouragement given the students to do free compositions or both. It is significant to note that the re-test scores of some of the students with relatively high I.Q.s whose scores were low in the first test have risen comparatively higher than those of the others. Gunawardhane of 9C provides an example."[4]

11.2 The experience was similar at the A+ category of school, Govt. Tamil School, Peradeniya. At the second administration of Test I, the A.M. was 41 (S.D. 18). The school has very inadequate facilities, and the pupils' conditions were very difficult. Nevertheless the increase was one of 9. In the case of Test I, 5 out of the 12 students got marks over 35, but in the second administration 6 out of 12 scored over 35 ; though this does not indicate much, the highest in the second administration was 73 while in the first administration it was only 50. The teacher reports that of the 12 students in the class, 3 students had irregular attendance which is also responsible for the slower improvement in this A+ category of school.

11.3 At Mowbray the course was done for one full term in Grade 8 on the class teacher's own initiative. There were no interruptions as was the case when the diploma in education students took the classes. The result was an A.M. of 56.2, in Test II a higher mean than for Grade 9 which was taken by the diploma-in-education student. Only 4 students out of the 36 students in class scored less than 35%.

11.4 At Girls' High School the Grade 9 was a Tamil medium class with 22 Arts (generally known to be the weaker streams in schools) and 15 science students. This group did the school's own December term test which was given to all the other parallel Grade 9 classes. The best of these other Grade Nines was the Sinhala medium 9A (Science) class (the control group). At the common term test in December the Grade 9 Tamil medium, after its roughly six month *Reconstruction Course*, scored an A.M. of 52.1, while the Sinhala medium class scored an A.M. of 50.3. This in itself is not a significant difference. 7 scored below 35 in the Sinhala medium Grade 9A, and 13 above 50, but in our experimental class though there were 9 below 35, *there were* 20 *above* 50.

12. *Conclusions*

The consistently better performance after use of the reconstruction teaching material shows that the technique of meaning-conveying through the mother tongue ensured better learning of English. It also incidentally indicated that other factors in the course, such as the gradations, psychological factors of motivation and interest, as well as repetition techniques, all of which were built into the Course that was tried out may have helped. But the chief factor was no doubt meaning-conveying in the mother tongue, both Sinhala and Tamil. Every teacher commented on the improvement, and it may do to conclude with two quotations :

> (*a*) " The result of the second test shows an improvement in application, word-order, and the mechanical skills such as spacing, full-stops, capitals etc.
>
> In the first test Mahindaratna's performance in word order exercises was very poor. Only one out of five attempts was successful. In the second test he was successful in three out of five attempts. Of the total number of 31 pupils 18 knew the word order of 4 out of 5 sentences. In the application exercises 14 out of 31 were able to write more than 5 sentences perfectly. They made no mistakes in tense, word order, spelling, spacing of words, capitals, etc. Some examples of sentences written are :

1. The farmer winnows the paddy with a fan and separates the grain from the chaff.
2. They take the sheaves of paddy to the threshing floor.
3. They tap the bark of the rubber trees."[1]

(*b*) "The explanation for the improved performance is mainly the better acquisition and retention of sentence meaning which was made possible by the Reconstruction Technique. Dodson says 'No matter whether a foreign language is learnt in the class room, at home or in the foreign country, the learner must first of all acquire the meaning of the sentence he hears before he can respond in speech or action'...... Pictures were not used and the mother tongue as a meaning-conveyor worked very well with the students...... This explains the reason why the students performed so well in Test 2"[9]

13. *Drawbacks.*

The drawbacks of this investigation were :

(*a*) The inability to carry on the teaching project for more than 6 to 8 weeks under one teacher, as the diploma-in-education teachers had to hand over to the class teachers who were themselves hesitant abous going on with the experiment with "full steam" because of syllabuset sent out by the Ministry of Education. Thus the experiment was not conducted under the most favourable conditions for the new technique.

(*b*) The fear of co-operation on the part of the schools, not because of unwillingness but because of the stranglehold of the text books — mainly a misconception no doubt, for in Colombo a Circuit Education Officer in English is openly advocating rejection or ignoring of the text books.

(*c*) The absence of a secondary school run by the University which could be a laboratory for curricular experiments in the teaching of the arts, languages, and the sciences.

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Appendix I

ANALYSIS OF THE ENGLISH ATTAINMENT TESTS

TEST I

SKILLS EXAMINED		RECOGNITION		RECALL		COMPOSITION		COMPREHENSION	
		Items	*Marks*	*Items*	*Marks*	*Items*	*Marks*	*Items*	*Marks*
	1. Alphabet	—	—	5	5	—	—	—	—
	2. Spelling	—	—	5	5	—	—	—	—
	3. Sounds	—	—	5	5	—	—	—	—
	4. Vocabulary	5	5	—	—	—	—	—	—
	5. Vocab. & Spelling	—	—	5	10	—	—	—	—
Grammatical Structure	6. Plural of nouns	—	—	5	5	—	—	—	—
	7. Word Order	—	—	3	3	—	—	—	—
	8. Either Word Order/ Vocab./ Tense Formation/Spelling	—	—	4	12	—	—	—	—
	9. Either Articles/Prep./ Tense Formation	—	—	6	8	—	—	—	—
10. Composition		—	—	—	—	4	16	—	—
11. Comprehension		—	—	—	—	—	—	3	6
Total No. items (50)		5		38		4		3	
Total Marks (80)			5		53		16		6

TEST II

SKILLS EXAMINED		RECOGNITION		RECALL		COMPOSITION		COMPREHENSION	
		Items	*Marks*	*Items*	*Marks*	*Items*	*Marks*	*Items*	*Marks*
Grammatical Structure	Plural	—	—	2	2	—	—	—	—
	Word Order	—	—	6	6	—	—	—	—
	Tense Formation	—	—	5	5	—	—	—	—
	Prepositions	—	—	3	3	—	—	—	—
	Articles	—	—	4	4	—	—	—	—
	Pronouns	—	—	3	3	—	—	—	—
	Negative	—	—	2	2	—	—	—	—
Composition		—	—	—	—	10	40	—	—
Comprehension									
	Q. 1 I.M.	—	—	—	—	—	—	1	3
	Q. 2 U.R.	—	—	—	—	—	—	1	6
	Q. 3 I.M.	—	—	—	—	—	—	1	2
	Q. 4 I.M.*	—	—	—	—	—	—	1	2
	Q. 5 U.R.	—	—	—	—	—	—	1	2
Total No. items (40)		—		25		10		5	
Total Marks (80)			—		25		40		15

Appendix II

"The Arithmetic Means of the second test and first test which are 50.7 and 46.2 respectively when compared show an overall improvement of 4.5. This is a meagre improvement but the two tests are of two different types as stated earlier. [Appendix I]

Whilst the earlier test was a more easy one with questions of the recognition type as well as the recall type the second had questions only of the recall type as the analysis of the tests have made clear. A better comparison would be that of the averages scored for the recall type of questions in the first paper with that of the marks scored in the second test. The average scored for the recall type of questions in this test is only 19.6% whilst in the second test it is 50.7%. Thus a marked improvement of about 31.1 is clearly seen."

(From : The report of Miss M. C. Perera, Mahamaya College).

Appendix III

"*The amount of time devoted to the teaching* A period of 4 weeks beginning from 3rd July to 3rd August— 30 periods of 40 mins. each were devoted to this.

Tabulated below are the results of a test held at the end of this period.

Part A : Grammatical structure.

Area or skill tested	*Percentage of correct answers*
Recall of tense forms	87
Negative forms	78.8
Number	87.8
Word order	95.7
Prepositions	96
Articles	78.8
Pronouns	100

In *Parts B and C* (comprehension and composition) the results are presented in a form which enables comparison with the 1st attainment test given at the beginning of the course.

	1st test	*2nd test*
Comprehension I.M. only	76.2%	92.0%
Comprehension taken as a whole	76.2%	88.4%
Composition	31.4%	83.3%

The highest number of mistakes in the objective question were in the use of prepositions. There is a very strong probability that students were misled by the ' format ' of the questions 19—22, not realising that the three sentences were to be taken as a paragraph and not as individual sentences.

It is also possible that a few students were unaware that ' are ' is a form of the verb ' to be ' — though they may have been familiar with the verbs ' are ' and ' is ' as they are.

100% performance by all the students was the aim of this course and it could be achieved with more practice and drilling.

Part B Comprehension — The performance compares favourably with the performance in the earlier attainment test which for comprehension had simple I.M. questions only.

The second test had 3 more difficult I.M. questions which fall into category U.R. The vocabulary in the passage given did not reflect the vocabulary used in the course.

Part C Composition — There was a choice given, but it was rather unbalanced because students had the option of choosing between application in the form of Recall or free Response, and many opted for the recall. The performance in the 2nd test shows a great deal of improvement.

There has been improvement in all spheres, the most remarkable being in the skill of free composition. The scores on this test have :

A.M. — 85.18
S.D. — 14.7

The difference in Mean between the attainment test and the achievement test — 30.9

The greatest frequency on this test is *between* 94-100, and only 3 *scores fall below the former mean of* 54.28. The greatest frequency *on the* 1*st test was in the class interval* 48-40 and *only* 4 *students had scores over* 70."

(*From* : The report of Mrs. D. A. Rajasena, Girls' High School, Kandy).

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SHORT COMMUNICATION

A Method for Assay of Total Potential Cyanide in Manioc Flour

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Manioc and its products usually contain small amounts of free cyanide and larger amounts of 'bound' cyanide. 'Bound' cyanide occurs mainly in the form of cyanogenic glucosides. Any method of determination of cyanogenic glucosides in plant material consists of three stages :

(1) Hydrolysis of the cyanogenic glucoside to give hydrogen cyanide (Liberation).
(2) Isolation of hydrogen cyanide (Recovery).
(3) Quantitation of the hydrogen cyanide.

Methods for determination of the cyanogenic glucosides (linamarin and lotaustralin) in manioc have been reported as early as 1944[2]. Nearly all the early methods relied either on acid hydrolysis or spontaneous autolysis by the endogenous enzyme linamarase, contained in the plant tissue, to liberate the bound cyanide[5]. These methods have been found wanting because linamarin is not readily hydrolysed by acid and because there is generally insufficient endogenous enzyme. Although Wood[7], using a model system of purified glucosides and linamarase, obtained a recovery of 87% after steam distillation, the critical factors that determine the reproducibility of the technique, *viz*, the total release and isolation of the cyanide and the prevention of losses due to secondary reactions have received insufficient attention.

For the development of a standard and uniform analytical procedure, factors including sample size, pH, addition of exogenous linamarase, time of incubation, recovery by aspiration with air or steam distillation and the effect of temperature and time on recovery, were studied. After liberation and isolation, cyanide was quantitatively determined by the colorimetric picric acid method[4,6].

Manioc was processed into flour by the traditional method of chipping, washing, drying and grinding. Samples (10 g) of this flour (moisture content 10.0%), were used for the assays.

The rate of distillation and volume of distillate for maximum recovery of liberated cyanide were determined for both aspiration with air and steam distillation. Aspiration was found to be less efficient than steam distillation (*Table* 1).

Table 1. Comparative recovery of HCN by steam distillation and aspiration with air

Method of hydrolysis	Maximum Recovery (HCN liberated, ppm)		Relative efficiency of aspiration
	Steam distillation	*Aspiration with air*	
Endogenous enzyme only (2 h)	49	36	0.73
With added exogenous enzyme (2 h)	112	81	0.71
$2N\ H_2SO_4$	57	39	0.68

Table 1 also shows that hydrolysis by the endogenous enzyme, or by acid is relatively inefficient. This point is further illustrated in the next two tables. *Table* 2 shows that hydrolysis by the endogenous linamarase requires the use of long incubation times. *Table* 3 shows that acid hydrolysis results in only one third to half the yield obtained with the exogenous enzyme. This data is compatible with studies on the acid hydrolysis of pure linamarin.[1] Use of an acid medium for distillation appears to give slightly lower values of cyanide ; this is similar to an observation by Zitnak[8].

Table 2. HCN liberated by endogenous enzyme

Weight of sample (g)	Time of incubation (h)	HCN liberated (ppm)
20	2	33
10	2	39
20	18	93
20	24	93

Table 3. HCN recovered (ppm) under different conditions

Hydrolysis with 2*N* H_2SO_4	Hydrolysis with added exogenous enzyme	
	Distillation at tissue pH	*Distillation in 2N acid medium*
59	157	156
65	140	145
57	146	132
	156	143
	163	140

The efficiency of the method was tested by recovery of added linamarin (instead of the added cyanide as done previously[3]). Recoveries of 88 — 90% were obtained. A further 5 — 6% can be obtained by a two step process i.e. distillation after the action of exogenous enzyme, followed by addition of 2*N* acid and re-distillation.

Unsieved manioc flour was used in the above assays. On sieving, much better reproducible results were obtained ; a sample of sieved manioc flour yielded values of 140, 142 and 141 ppm cyanide in successive assays. Free cyanide in the flour used was of the order of 5 ppm.

In view of the results obtained, the following method can be recommended for the determination of total potential cyanide in manioc flour.

Manioc flour (10 g) is introduced into a distillation flask, water (200 ml) and excess linamarase (approx. 450 μg/min.) is then added. The sample is incubated for 2h. at room temperature and distilled ; 250 to 300 ml of distillate is collected in a solution of 0.5g sodium hydroxide in 50 ml of water. Cyanide is then estimated by the colorimetric picric acid method[6].

The assay of total potential cyanide in other forms of processed and unprocessed manioc is in progress.

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